

FORM PTO-1390 (Modified) (REV 11-98)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER <b>Bayer 10,131-KGB</b>
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR <b>09/786635</b>		
INTERNATIONAL APPLICATION NO. <b>PCT/EP99/06991</b>	INTERNATIONAL FILING DATE <b>21 September 1999 (21.09.99)</b>	PRIORITY DATE CLAIMED <b>25 September 1998 (25.09.98)</b>		
TITLE OF INVENTION <b>ATP BINDING CASSETTE GENES AND PROTEINS FOR DIAGNOSIS AND TREATMENT OF LIPID DISORDERS AND INFLAMMATORY DISEASES</b>				
APPLICANT(S) FOR DO/EO/US <b>SCHMITZ, Gerd and KLUCKEN, Jochen</b>				
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:				
<ol style="list-style-type: none"> <li><input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li><input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li><input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2))             <ol style="list-style-type: none"> <li><input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li><input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li><input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li><input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).</li> <li><input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))             <ol style="list-style-type: none"> <li><input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input type="checkbox"/> have been transmitted by the International Bureau.</li> <li><input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li><input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li><input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li><input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).</li> <li><input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).</li> <li><input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> </ol>				
Items 13 to 20 below concern document(s) or information included:				
<ol style="list-style-type: none"> <li><input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li><input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li><input type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li><input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li><input type="checkbox"/> A substitute specification.</li> <li><input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li><input checked="" type="checkbox"/> Certificate of Mailing by Express Mail</li> <li><input type="checkbox"/> Other items or information:  <div style="border: 1px solid black; height: 100px; width: 100%;"></div> </li> </ol>				

U.S. APPLICATION NO. IF KNOWN, SEE 37 CFR  
**097786635**INTERNATIONAL APPLICATION NO.  
PCT/EP99/06991ATTORNEY'S DOCKET NUMBER  
Bayer 10,131-KGB

21. The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .....	\$1,000.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .....	\$860.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....	\$710.00
<input checked="" type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) .....	\$690.00
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) .....	\$100.00

**ENTER APPROPRIATE BASIC FEE AMOUNT =**

Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)).

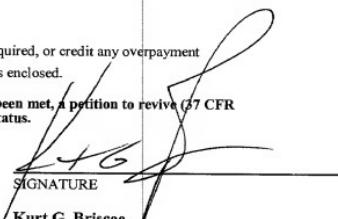
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Independent claims	5 - 3 =	2	x \$80.00	\$160.00
Multiple Dependent Claims (check if applicable).				\$270.00
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Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable).				<input type="checkbox"/> \$0.00
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<b>TOTAL NATIONAL FEE =</b>				<b>\$1,120.00</b>
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).				<input type="checkbox"/> \$0.00
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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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GT/GCT Read 7 MAR 2001

PC1/EF99/00991

## ATP binding cassette genes and proteins for diagnosis and treatment of lipid disorders and inflammatory diseases

#### **Background of the invention**

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Reverse cholesterol transport mediated by HDL provides a "protective" mechanism for cell membrane integrity and foam cell formation and cellular cholesterol is taken up by circulating HDL or its precursor molecules. The precise mechanism of reverse cholesterol transport however is currently not fully understood and the mechanism of cellular cholesterol efflux and transfer from the cell surface to an acceptor-particle, such as HDL, is yet unclear. Certain candidate gene products have been postulated playing a role in the process of reverse cholesterol transport [1]. Apolipoproteins (e.g. ApoA-I, ApoA-IV), lipid transfer proteins (e.g. CETP, PLTP) and enzymes (e.g. LCAT, LPL, HL) are essential to exchange cholesterol and phospholipids in lipoprotein-lipoprotein and lipoprotein-cell interactions. Different plasma membrane receptors, such as SR-BI [2; 3], HB1/2 [4], and GPI-linked proteins (e.g. 120 kDa and 80 kDa) [5] as well as the sphingolipid rich microdomains (Caveolae, Rafts) of the plasma membrane have been implicated being involved in the process of reverse cholesterol transport and the exchange of phospholipids. How these membrane-microdomains are organized is in the current focus of interest for the identification of therapeutic targets. In recent studies SR-BI function as receptor for uptake of HDL into the liver and steroidogenic tissues could be demonstrated and the effectivity of this process is highly dependent on the phospholipid environment [2].

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microdomains are organized is in the current focus of interest for the identification of therapeutic targets. In recent studies SR-BI function as receptor for uptake of HDL into the liver and steroidogenic tissues could be demonstrated and the effectivity of this process is highly dependent on the phospholipid environment [2].

25

Cholesterol and phospholipid homeostasis in monocytes/macrophages and other cells involved in the atherosclerotic process is a critical determinant in atherosclerotic vessel disease. The phagocytic function of macrophages in host defense, tissue remodelling, uptake and lysosomal degradation of atherogenic lipoproteins and membrane fragments or other lipid containing particles has to be balanced by effective release mechanisms to avoid foam cell formation. HDL mediated reverse

cholesterol transport, supported by endogenous ApoE and CETP synthesis and secretion provides an effective mechanism to release excessive cholesterol from macrophages and other vascular cells.

- 5 Alternatively, reduced cholesterol and triglyceride/fatty acid absorption by intestinal mucosa cells as well as increased lipid secretion from hepatocytes into the bile will lower plasma lipids and the concentration of atherosclerotic lipoproteins.

#### Summary of the invention

10

New cholesterol responsive genes were identified with differential display method in human monocytes from peripheral blood that were subjected to macrophage differentiation and cholesterol loading with acetylated LDL and subsequent deloading with HDL<sub>3</sub>.

15

- In an initial screen ABCG1 (ABC8), a member of the rapidly growing family of ABC (ATP-Binding Cassette) transport systems, that couple the energy of ATP hydrolysis to the translocation of solutes across biological membranes, was identified as a cholesterol sensitive switch. ABCG1 is upregulated by M-CSF dependent phagocytic differentiation but expression is massively induced by cholesterol loading and almost completely set back to differentiation dependent levels by HDL<sub>3</sub>.

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- In a more detailed analysis 37 already characterised ABC members and 8 Fragment - sequences (Table 2) were analysed in monocyte/macrophage cells by RT-PCR (linear range) for differentiation dependent changes and cholesterol sensitivity.

25

Among the 45 tested ABC-transporter genes 18 of the characterized ABC transporters and 2 of the Fragment -sequence based ABC-transporters are cholesterol sensitive (Example 4).

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The cholesterol sensitive ABC-transporter are named according to the new ABC-

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nomenclature and listed in Table 3 with the new and the old designations, respectively.

The most sensitive gene was ABCG1. ABCG1 is the human homologue of the drosophila white gene. Sequencing of the promoter of ABCG1 (Example 7) shows important transcription factor binding sites relevant for phagocytic differentiation and lipid sensitivity.

Antisense treatment of macrophages during cholesterol loading and HDL<sub>1</sub>-mediated deloading clearly identified ABCG1 as a cholesterol transporter and the efflux of choline-containing phospholipids (phosphatidylcholine, sphingomyelin) was also modulated. Northern- and Western blot analysis provided further support that inhibition of cholesterol transport is associated with lower ABCG1 mRNA expression and ABCG1 protein levels (Example 5).

Considerable evidence was derived from energy transfer experiments (Example 3) that ABCG1 in the cell membrane is in a regulated functional cooperation (e.g. cell differentiation, activation, cholesterol loading and deloading) with other membrane receptors that have either transport- (e.g. LRP-LDL receptor related protein) or signalling- and adhesion-function (e.g. integrins, integrin associated proteins) which is also supported by sequence homology of extracellular domains as well as other parts of the ABCG1 sequence. For example the protein sequence of the region of the third extracellular loop of ABCG1, i.e. aminoacid residues 580 through 644, shares homology with fibronectin (aa 317-327), integrin $\beta$ 5 (aa 538-547), RAP (aa 119-127), LRP (aa 2874-2894), apoB-100 precursor (aa 4328-4369), glutathion-S-transferase (aa 54-78) and glucose transporter (aa 371-380). Sequence comparison of all cholesterol sensitive transporters indicates this as a general principle of ABC transporter function and regulation.

Among the other cholesterol sensitive genes ABCA1 (ABC1) was further characterized. ABCA1 was identified in the mouse as an IL-1 $\beta$  transporter

involved also in apoptotic cell processing. We show here, by RT-PCR (Table 2) and confirmation by Northern analysis, based on the newly detected human ABCA1 cDNA sequence (Example 6), that ABCA1 follows the same regulation as ABCG1.

5 Moreover, the ABCA1-knockout mice (ABCA1<sup>-/-</sup>) show massively reduced levels of serum lipids and lipoproteins. The expression of ABCA1 in mucosa cells of the small intestine and the altered lipoprotein metabolism in ABCA1<sup>-/-</sup> mice allows the conclusion that ABCA1 plays a major role in intestinal absorption and translocation of lipids into the lymph-system

10 Analysis of genetic defects that affect macrophage cholesterol homeostasis identified dysregulated ABCA1 as a gene locus involved in the HDL-deficiency syndrome (Tangier-Disease). This disease is associated with hypertriglyceridemia and splenomegaly.

15 Another as yet not described HDL-deficiency syndrome associated with early onset of coronary heart disease and psoriasis showed a dysregulation of the chromosome 17 associated ABC-sequences (ABCC4 (MRP3); ABCC3 (MRP3); ABCA5 (Fragment 90625); ABCA6 (Fragment 155051) :17q21-24). This points to an association with the predicted gene locus for psoriasis at chromosome 17.

20 A recently sequenced human ABC-transporter (ABCA8, Example 9) shows high homology to ABCA1 and also belongs to the group of cholesterol sensitive ABC-transporter.

25 ABCC5 (MRP5, sMRP) is a member of the MRP-subfamily among which ABCC2 (MRP2, cMOAT) was characterized as the hepatocyte canalicular membrane transporter that is involved in bilirubin glucuronide secretion [9] and identified as the gene locus for Dubin-Johnson Syndrome [10] a disorder associated with mild chronic conjugated hyperbilirubinemia.

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Furthermore, the identification of ABCA1 as a transporter for IL-1  $\beta$  identifies this gene as a candidate gene for treatment of inflammatory diseases including rheumatoid arthritis and septic shock. The cytokine IL-1  $\beta$  is a broadly acting proinflammatory mediator that has been implicated in the pathogenesis of these

5 diseases.

Moreover, we could demonstrate, that glyburide as an inhibitor of IL-1  $\beta$  secretion inhibits not only Caspase I mediated processing of pro-IL-1  $\beta$  and release of mature IL-1  $\beta$  but simultaneously inhibits ceramide formation from sphingomyelin mediated by neutral sphingomyelinase and thereby releases human fibroblasts from G<sub>1</sub>-phase cell cycle arrest. These data provide a further mechanism indicative for a function of ABCA1 in signalling and cellular lipid metabolism.

10 Autoimmune disorders that are associated with the antiphospholipid syndrome (e.g. lupus erythematoses) can be related to dysregulation of B-cell and T-cell function, aberrant antigen processing, or aberrations in the asymmetric distribution of membrane phospholipids. ABC-transporters are, besides their transport function, candidate genes for phospholipid translocases, floppases and scramblases that regulate phospholipid asymmetry (outer leaflet: PC+SPM; inner leaflet: PS+PE) of 15 biological membranes [11]. There is considerable evidence for a dysregulation of the analysed ABC-transporters in patient cells. We conclude that these ABC-cassettes 20 are also candidate genes for a genetic basis of antiphospholipid syndromes such as in Lupus erythematoses.

25 In summary, the ABC genes ABCG1, ABCA1 and the other cholesterol-sensitive ABC genes as specified herein, can be used for diagnostic and therapeutic applications as well as for biochemical or cell-based assays to screen for pharmacologically active compounds which can be used for treatment of lipid disorders, atherosclerosis or other inflammatory diseases. Thus it is an objective of 30 the present invention to provide assays to screen for pharmacologically active compounds which can be used for treatment of lipid disorders, atherosclerosis or

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other inflammatory diseases. Further the invention provides tools to identify modulators of these genes and gene products. These modulators can be used for the treatment of lipid disorders, atherosclerosis or other inflammatory diseases or for the preparation of medicaments for treatment of lipid disorders, atherosclerosis or other inflammatory diseases. The medicaments comprise besides the modulator acceptable and usefull pharmaceutical carriers.

## Abbreviations

aa	Amino acid
ABC	ATP-binding cassette
ABCA#	ATP-binding cassette, sub-family A (ABC1), member #
ABCB#	ATP-binding cassette, sub-family B (MDR/TAP), member #
ABCC#	ATP-binding cassette, sub-family C (CFTR/MRP), member #
ABCD#	ATP-binding cassette, sub-family D (ALD), member #
ABCE#	ATP-binding cassette, sub-family E (OABP), member #
ABCF#	ATP-binding cassette, sub-family F (GCN20), member #
ABCG#	ATP-binding cassette, sub-family G (WHITE), member #
ABCR	Homo sapiens rim ABC transporter
AcLDL	Acetylated LDL
ADP1	ATP-dependent permease
ALDP	Adrenoleukodystrophy protein
ALDR	Adrenoleukodystrophy related protein
ApoA	Apolipoprotein A
ApoE	Apolipoprotein E
ARA	Anthracycline resistance associated protein
AS	Antisense
ATP	Adenosine triphosphate
CETP	Cholesteryl ester transfer protein
CFTR	Cystic fibrosis transmembrane conductance regulator
CGT	ceramide glucosyl transferase
CH	Cholesterol
cMOAT	Canalicular multispecific organic anion transporter
dsRNA	Double stranded RNA
Fragment	Gen Fragment
FABP	plasma membrane fatty acid binding protein

FACS	Fluorescence activated cell sorter
FATP	intracellular fatty acid binding protein
FCS	foetal calf serum
FFA	free fatty acids
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCN20	protein kinase that phosphorylates the alpha-subunit of translation initiation factor 2
GPI	Glycosylphosphatidylinositol
HaCaT	keratinocytic cell line
HDL	High density lipoprotein
HL	Hepatic lipase
HlyB	haemolysin translocator protein B
HMT1	yeast heavy metal tolerance protein
HPTLC	High performance thin layer chromatography
IL	Interleukin
LCAT	Lecithin:cholesterol acyltransferase
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
LRP	LDL receptor related protein
MDR	Multidrug resistance
MRP	Multidrug resistance-associated protein
PC	Phosphatidylcholine
PE	Phosphatidylethanolamin
PL	Phospholipid
PLTP	Phospholipid transferprotein
PMP	peroxisomal membrane protein
PS	Phosphatidylserine
RNA	Ribonucleic acid
RT-PCR	Reverse transcription – polymerase chain reaction
SDS	Sodium dodecyl sulfate

SL	Sphingolipid
sMRP	Small form of MRP
SPM	Sphingomyelin
SR-BI	Scavenger receptor BI
SUR	Sulfonylurea receptor
TAP	Antigen peptide transporter
TG	Triglycerides
TSAP	TNF-alpha stimulated ABC protein
UTR	untranslated region

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**Description of the Figures**

Figures 1 to 5 are showing nucleotide and protein sequences described in this application. The sequences are repeated in the sequence listing.

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**Description of Tables:****Table 1:**

Levels of RNA transcripts of ABCG1 (ABC8), ABCA1 (ABC1) and ABCA8 in human tissues were determined by Northern blot analysis of a multiple tissue dot-blot (Human RNA MasterBlot, Clontech Laboratories, Inc., CA, USA). The relative amount of expression is indicated by different numbers of filled circles.

**Table 2:**

The expression pattern of ABC-transporters in monocytes, monocyte derived macrophages (3 days cultivated monocytes in serum free Macrophage-SFM medium containing 50 ng/ml M-CSF), AcLDL incubated monocytes (3 days with 100 µg/ml) followed by HDL<sub>3</sub> (100 µg/ml) incubated monocytes is shown. Expressed genes are tested for cholesterol sensitivity by semiquantitative PCR.

For known ABC-Transporter the chromosomal location and the transported molecules are also presented.

**Table 3:**

Disorders, that are associated with ABC-transporters are shown. The chromosomal location is indicated and the relevant accession number in OMIN (Online Mendelian Inheritance in Man).

**Table 4:**

Expression of ABC-Transporters in HaCaT keratinocytic cells during differentiation

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Table 1

<i>Tissue</i>	ABCG1 (ABC8)	ABCA1 (ABC1)
Adrenal gland	•••••	•••
Thymus	••••	••
Lung	••••	•••
Heart	•••	••
Skeletal	••	•
Brain	•••	••
Spleen	•••••	••
Lymphnode	•••	•
Pancreas	•	•
Placenta	•••••	•••••
Colon	••	•
Small intestine	••	•••••
Prostate	••	•
Testis	•	•
Ovary	••	•
Uterus	•	••
Mammary gland	••	•
Thyroid gland	••	••
Kidney	••	•
Liver	•••	•••
Bone marrow	•	•
Peripheral leukocytes	•	•
<i>Fetal tissue</i>		
Fetal brain	•	••
Fetal liver	•	••••
Fetal spleen	••	•••
Fetal thymus	••	••
Fetal lung	••	•••

Table 2: Cholesterol dependent gene regulation of human ABC transporters

Gene	chromosomal localization	peripheral blood monocytes	3 days old M-CSF MØ	cholesterol loading (acLDL)	cholesterol deloading (HDL3)	transported molecules
ABCG1 (ABC8)	21q22.3	+	↑	↑↑	↓↓	cholesterol / choline PL
ABCA1 (ABC1)	9q22-31	+	↑	↑↑	↓↓	cholesterol / IL-1Ø
ABCC5 (MRP5)	3q25-27	+	↑	↑↑	↓	
ABCD1 (ALDP, ALD)	Xq28	+	↑	↑	↓	very long chain fatty acids
ABCA5 (est90625)	17q21-25	+	↑	↑	↓	
ABCB11 (BSEP, SPGP)	2q24	+	↑	↑↑	↓	bile acids
ABCA8 (ABC-new)		+	+	↑	↓	
ABCC2 (MRP2)	10q23-24	+	+	↑	↓	bilirubin glucuronide
ABCB6 (est45597)	2q33-36	+	+	↑	↓	
ABCC1 (MRP1)	16p13.12	+	↓	↑	↓	eicosanoids
ABCA3 (ABC3)	16p13.3	+	↑	↑	nr	
est1133530		+	↑	↑	nr	
ABCB4 (MDR3)	7q21	+	↑	↓	↑	phosphatidylcholine
ABCG2 (est157481, ABCP)	4q22-23	+	↑	↓	↑	
ABCC4 (MRP4)	13q31	+	↑	↓	↑	
ABCB9 (est122234)	12q24	+	↑	↓	↑	
ABCD2 (ALDR)	12q11	+	↓	↓	↑	very long chain fatty acids
ABCB1 (MDR1)	7q21	+	+	↓	↑	phospholipids, amphiphiles
ABCA6 (est155051)	17q21	+	↑	↓	nr	
est640918		+	↑	↓	nr	
ABCD4 (P70R)	14q24.3	+	↑	nr	nr	
ABCA2 (ABC2)	9q34	+	↑	nr	nr	
ABCF2 (est133090)	7q35-36	+	↑	nr	nr	
ABCB7 (ABC7)	Xq13.1-3	+	↑	nr	nr	iron
ABCF1 (ABC50, TSAP)	6p21.33	+	↑	nr	nr	
ABCC6 (MRP6)	16p13.11	+	↓	nr	nr	
ABCB5 (est422562)	7p14	+	↓	nr	nr	
ABCC3 (MRP3)	17q11-21	+	nr	nr	nr	
ABCA4 (ABCR)	1p22	+	nr	nr	nr	retinoids, lipofuscin
ABCB2 (TAP1)	6p21.3	+	nr	nr	nr	peptides
ABCB3 (TAP2)	6p21.3	+	nr	nr	nr	peptides

Gene	chromosomal localization	peripheral blood monocytes	3 days old M-CSF MØ	cholesterol loading (acLDL)	cholesterol deloading (HDL3)	transported molecules
ABCF3 (est201864)	3q25.1-2	+	nr	nr	nr	
ABCB8 (est328128)	7q35-36	+	↑	nr	nr	
ABCE1 (OABP)	4q31	+	↑	nr	nr	
ABCB10 (est20237)	1q32	+	↑	nr	nr	
est698739		+	↑	nr	nr	
ABCC10 (est182763)	6p21	+	nr	nr	nr	
ABCC7 (CFTR)	7q31	∅	∅	∅	∅	ions
ABCC8 (SUR-1)	11p15.1	∅	∅	∅	∅	
ABCD3 (PMP70)	1p21-22	∅	∅	∅	∅	
Huwhitc2		∅	∅	∅	∅	
est1125168		∅	∅	∅	∅	
est1203215		∅	∅	∅	∅	
est168043		∅	∅	∅	∅	
est990006		∅	∅	∅	∅	

+ = expressed

∅ = not expressed

nr=not regulated

↑ = upregulated

↓= downregulated

half (hs) or full size (fs) transporter as deduced from the mRNA size

**Table 3**

<i>Disorders</i>	<i>Genomic location</i>	<i>Associated gene</i>	<i>OMIM-acc.nr.</i>
<i>Metabolic disorders:</i>			
Cystic fibrosis	7q31.3	ABCC7 (CFTR)	219700
Dubin Johnson syndrome (mild chronic conjugated hyperbilirubinemia)	10q24	ABCC2 (CMOAT)	237500
Progressive familial intrahepatic cholestasis type III (PIFC3)	7q21.1	ABCB4 (MDR3)	602347
<i>Byler disease (PFIC2)</i>	<i>2q24</i>	<i>ABCB11 (BSEP, sPGP)</i>	<i>601847</i>
Familial persistent hyperinsulinemic hypoglycemia	11p15.1	ABCC8 (SUR-1)	601820
IDDM	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	222100
<i>Neuronal disorders:</i>			
Adrenoleukodystrophy	12q11	ABCD2 (ALDR)	300100
Zellweger's syndrome	1p22-21	ABCD3 (PMP70)	214100
Multiple Sclerosis	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	126200
X-linked Sideroblastic anemia with spinocerebellar ataxia	Xq13.1-3	ABCB7 (ABC7)	301310
Menkes disease (altered homeostasis of metals)	Xq13	ABCB7 (ABC7)	309400
<i>Immune/Hemostats disorders:</i>			
Herpes simplex virus infection [12]	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	
Behcet's syndrome	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	109650
Bare lymphocyte syndrome type I	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	209920
Scott syndrome	7q21.1	ABCB1 (MDR1)	262890
<i>Retinal dystrophies:</i>			
Fundus flavimaculatus with macular dystrophy	1p13-21	ABCA4 (ABCR)	601691
Juvenile Stargardt disease	1p13-21	ABCA4 (ABCR)	248200
Age-related macular degeneration	1p13-21	ABCA4 (ABCR)	153800
Cone-rod dystrophy	1p13-21	ABCA4 (ABCR)	600110
Retinitis pigmentosa	1p13-21	ABCA4 (ABCR)	601718

<i>Diseases with evidence for involvement of ATP cassettes/translocases and floppases[80]</i>	<i>Assumed gene</i>		
BRIC (Benign recurrent intrahepatic obstructive jaundice)	18	Assumed	243300
Psoriasis	17q11-12 17q21-24	ABCA5 (Fragment 90625) ABCC3 (MRP3)	602723 177900 601454
Lupus erythematoses – Antiphospholipid Syndrome		Translocase Flippase	152700
PFIC (Prog. Fatal familial intrahepatic cholestasis)	PFIC1	18q21-22	ATP Transporters
<i>Neurological disorders mapped to gene locus of ABCG1 (ABC8)</i>			
Autosomal bipolar affective disorder	21q22.3	ABCG1 (ABC8)	125480
Autosomal recessive non-syndromic deafness	21q22.3	ABCG1 (ABC8)	601072
Down Syndrome (ABC-8 may be a candidate for the Brushfield spots – mottled, marble or speckled irides frequently seen in Down-Syndrome)	21q22.3	ABCG1 (ABC8)	190685
Linkage to phosphofructokinase (liver type)	21q22		171860
<i>HDL-deficiency syndromes,</i> Gen responsible for Tangier Disease	9q31	ABCA1 (ABC1)	205400

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Table 4: Expression of ABC-Transporters in HaCaT keratinocytic cells during differentiation

Gene	chrom. localisation	initial expression	differentiation dependent expression	known or putative molecules transported
ABCG1 (ABC8)	21 q22.3	+++++	↑	cholesterol choline-PL
ABCC3 (MRP3)	17 q11-q12	+++++	↑	
ABCA8	19 p13	+++++	↑	
ABCC1 (MRP1)	16 p13	+++++	↗ ↘ (max. day 2)	PGA <sub>2</sub> , LTC <sub>4</sub> DNP-SG
ABCD4 (PMP69, P70R)	14 q24	+++++	↗ ↘ (max. day 2.4)	
ABCC2 (MRP2)	10 q24	+++	↗ ↘ (max. day 2)	bilirubin glucuronide
ABCA3 (ABC3)	16 p13	+	↗ ↘ (max. day 4.6)	
ABCA5 (ABCR)	1 p21	+	↗ ↘ (max. day 4)	retinoid, lipofuscin
ABCA1 (ABC1)	9 q22-q31	+	↗ ↘ (max. day 6)	
ABCC6 (MRP6)	16 p13.11	+	↗ ↘ (max. day 4)	
ABCC4 (MRP4)	13 q31	++++	↗ ↘ (max. day 2.4)	
ABCA2	9 q34	++++	↗ ↘ (max. day 6)	
ABCC5 (MRP5, SMRP)	3 q27	+++++	↗ ↘ (max. day 2.4)	

<b>ABCB6</b> (est45597)	2	+++++	↗ ↘ (max. day 2,4)	
<b>ABCB7 (ABC7)</b>	X q13.1-3	+++++	↗ ↘ (max. day 4)	irons
<b>TAP1 (ABCB1)</b>	6 p21.3	+++++	↗ ↘ (max. day 4,6)	peptides
<b>TAP2 (ABCB2)</b>	6 p21.3	+++++	↗ ↘ (max. day 2,4)	
<b>ABCB8</b> (est328128)	7 q35-36	+++++	↗ ↘ (max. day 2 )	
<b>EST640918</b>	17 q24	+	↗ ↘ (max day 4)	
<b>ABCC7 (CFTR)</b>	7 q31	+++	↗ ↘ (max day 4)	
<b>ABCB10</b> (est20237)	1 q32	+++	↗ ↘ (max. day 2)	
<b>ABCF1 (TSAP)</b>	6 p21.33	+++++	↓	
<b>ABCC10</b> (est182763)	1 q32	+++++	↓	
<b>ABCE1 (OABP)</b>	4 q31	+++++	↓	
<b>EST698739</b>	17 q24	+++++	↓	
<b>ABCF2</b> (est133090)	7 q35-q36	+++++	↓	
<b>ALD</b> (ABCD1,ALDP)	X q28	++++)	↓	VLCFA
<b>ABCA5</b> (est90625)	17 q21-q24	+++	↓	
<b>ABCB5</b> (est422562)	7 p14	++++	↓	
<b>ABCB9</b> (est122234)	12 q24-qter	++	↓	
<b>ABCD2 (ALDR)</b>	12 q11	+	↓	VLCFA
<b>ABCF3</b> (est201864)	3 q25.1-2	+++++	↓	
<b>ABCG2 (ABC15,ABCP)</b>	4 q22-q23	++++	↓	
<b>EST1133530</b>	4 p16pter	+++++	↓	

<b>Huwhite</b>	11 q23	+++++	↓	
ABCA6 (est155051)	17 q21	++	↓	
BSEP (ABCB11,SPGP)	2 q24	+	↓↑ (max day 6 )	
ABCB4 (MDR3)	7 q21	not expressed		phosphatidyl-choline
ABCD3 (PMP70)	1 p22	not expressed		
ABCB1 (MDR1)	7 q21	not expressed		phospholipids amphiphiles
EST168043	2 p15-16	not expressed		
EST990006	17 q24	not expressed		
ABCC8(SUR1)	11 p15.1	not expressed		

+ relative expression    n.d. not determined

↑: upregulated    ↓: downregulated    ↗↘: biphasic expression

**Description of specific embodiments****Candidate gene identification during cholesterol loading and deloading of human monocyte derived macrophages**

5

In order to discover genes that are involved in the cholesterol loading and/or deloading in vitro assays were set up. Particularly, gene expression in human blood derived monocytes and macrophages elicited by cholesterol and its physiological transport formulation, i.e. various low density lipoprotein (LDL) particle species like AcLDL, was studied.

10

Elutriated human monocytes were cultivated in M-CSF containing but serum free macrophage medium supplemented with AcLDL (100 µg protein/ml medium) for three days, followed by cholesterol depletion replacing AcLDL by HDL<sub>3</sub> (100 µg protein/ml medium) for twelve hours. Differential display screening for new candidate genes, regulated by cholesterol loading/deloading, was performed (Example 1).

**Identification of a new cholesterol sensitive gene**

20

ABCG1 (ABC8) was discovered as a novel cholesterol sensitive gene. ABCG1 belongs to the ATP binding cassette (ABC) transporter gene family. ABCG1 was recently published as the human analogue of the drosophila white gene [6-8].

25

The gene is strongly upregulated by AcLDL-mediated cholesterol loading, and almost completely downregulated by HDL<sub>3</sub> mediated-cholesterol deloading, as confirmed by Northern blot (Example 2). Northern blot analysis of mRNA from human monocyte-derived macrophages obtained from the peripheral blood probands clearly show upregulation of ABCG1 mRNA formation upon AcLDL incubation. In sharp contrast, ABCG1 mRNA expression was decreased in such macrophages upon incubation with HDL<sub>3</sub> containing medium.

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**ABCG1 expression in cholesterol loaded and deloaded cells after four days pre-differentiation**

- 5 For effective cholesterol loading monocytes must be differentiated to phagocytic-macrophage like cells. During this period scavenger receptors are upregulated and promote AcLDL uptake leading to cholestryl ester accumulation. After four days preincubation period we have incubated the cells for one, two and three days with AcLDL (100 µg/ml) to show cholestryl ester accumulation. After two days of loading we deloaded the cells with HDL<sub>3</sub> for 12 hours, 24 hours and 48 hours, respectively. ABCG1 is time dependently upregulated during the AcLDL loading period and downregulated by HDL<sub>3</sub> deloading (Examples 2 and 3) In order to confirm time dependent increase of ABCG1 mRNA expression after AcLDL challenge in human monocyte derived macrophages, Nothern blot analyses for ABCG1 mRNA quantification were made, RNA samples from the macrophages were harvested at day zero and day four as controls and mRNA samples were taken one, two, and three days after AcLDL treatment of macrophages. which started at day four. A dramatic increase of ABCG1 mRNA content of the macrophages could be detected from day five through day seven by Nothern blot analyses.
- 10
- 15
- 20 This regulation shows the same pattern as changes of cellular cholestryl ester content (Example3). Cholesterol ester accumulation starts in monocyte-derived macrophages upon AcLDL stimulation from a base level below 5 nmol/mg cell protein at day four up to 120 nmol/mg cell protein at day seven (i.e. three days after AcLDL application).
- 25

**Tissue expression**

- 30 Besides cholesterol loaded macrophages ABCG1 is prominently expressed in brain, spleen, lung, placenta, adrenal gland, thymus and fetal tissues (Table 1).

**Chromosomal location and associated genes and diseases**

The ABCG1 gene maps to human chromosome 21q 22.3. Also localized in this region 21q 22.3 are the following genes: integrin β 2 (CD18), brain specific polypeptide 19, down syndrome cell adhesion molecule, dsRNA specific adenosine deaminase, cystathionine β synthase, collagen VI alpha-2, collagen XVIII alpha-1, autosomal recessive deafness, and amyloid beta precursor.

This chromosomal region is in close proximity to other regions involved in Down syndrome, autosomal dominant bipolar affective disorder, and autosomal recessive non-syndromic deafness.

**Extracellular loop of ABCG1 (ABC8) for antibody generation**

The putative structure of the hydrophobic transmembrane region of ABCG1 shows 6 transmembrane spanning domains, and 3 extracellular loops, two of them are 9- and 8-amino acids-long, respectively, while the third one is 66-amino acids-long.

The larger one of the two intracellular loops consists of 30 amino acids. Similarity-survey in protein databases for homologies the 3rd extracellular loop (IIIex) with other genes resulted in the identification of fibronectin, integrin $\beta$ 5, RAP, LRP (LDL receptor related protein) apo-lipoprotein B 100 precursor protein, glutathion S-transferase and glucose transporter.

A polyclonal antiserum was generated against the 3rd extracellular loop (IIIex) of ABCG1 in order to perform flow cytometric analysis, energy transfer experiments and Western-blotting (see Example 3). In the amino acid sequence of ABCG1 the 3rd extracellular loop (IIIex) comprises 66 amino acids comprises 66 amino acids from amino acid 580 through 644. The peptide fragment for antibody generation comprises the amino acid residues 613 through 628 of ABCG1 polypeptide. ABCG1 obviously interacts with endogenous sequence motifs with other membrane receptors

involved in transport (e.g. LRP, RAP), signalling and adhesion (e.g. integrins, integrin associated proteins) as a basis of ABCG1-function and regulation. Moreover sequence comparisons of all ABC-transporters listed in Table 3 indicates functional cooperation with other membrane receptors as a general principle of the whole gene family.

### Subfamily-Analysis

Evolutionary relationship studies with the whole ABC transporter family have shown that ABCG1 (ABC8) forms a subfamily together ABCG2 (est157481) and this subfamily is closely related to the full-size transporters ABCA1 (ABC1), ABCA2 (ABC2), ABCA3 (ABC3), ABCA4 (ABCR) and the half-size transporter ABCF1 (TSAP).

Recent studies by Allikmets et al. have identified 21 new genes as ABC transporters by expressed sequence tags database search [13].

### General description of the ABC transporter family

The ATP-binding cassette (ABC) transporter superfamily contains some of the most functionally diverse proteins known. Most of the members of the ABC family (also called traffic ATP-ases) function as ATP-dependent active transporters (Table 3). The typical functional unit consists of a pair of ATP-binding domains and a set of transmembrane (TM) domains. The TM-domains determine the specificity for the type of molecule transported, and the ATP-binding domains provide the energy to move the molecule through the membrane [14; 15]. The variety of substrates handled by different ABC-transporters is enormous and ranges from ions to peptides. Specific transporters are found for nutrients, endogenous toxins, xenobiotics, peptides, aminoacids, sugars, organic/inorganic ions, vitamins, steroid hormones and drugs [16; 17].

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**ABC-transporter associated diseases**

The search for human disease genes (Table 3) provided a number of previously undiscovered ABC proteins [16]. The best characterized disease caused by a mutation in an ABC transporter is cystic fibrosis (ABCC7 (CFTR)). Inherited disorders of peroxisomal metabolism as Adrenoleukodystrophy and Zellweger's syndrome also show alterations in ABC transporters. They are involved in peroxisomal beta-oxidation, necessary for very long chain fatty acid metabolism [18].

**10 Antisense against ABCG1 inhibits cholesterol efflux to HDL<sub>3</sub>**

Since ABCG1 is a cholesterol sensitive gene and other ABC transporters are known to be involved in certain lipid transport processes, the question arises whether ABCG1 plays a role in transport of cholesterol, phospholipids, fatty acids or glycerols. Therefore antisense experiments were performed to test the influence of ABCG1 on lipid loading and deloading. The inhibition of ABCG1 with specific antisense oligonucleotides decreased the efflux of cholesterol and phosphatidyl-choline to HDL<sub>3</sub>. (Example 5)

**20 Other cholesterol sensitive ABC transporter**

Cloning and sequencing of the human ABCA1 (ABC1) provided the information to characterize ABCA1 for cholesterol sensitivity, and tissue distribution (Example 6). Another cholesterol sensitive human ABC transporter (ABCA8 ) has been cloned and sequenced (Example 8)

**Characterization of the ABCG1 promoter region**

The ABCG1 promoter has the characteristic binding sites for transcription factors that are involved in the differentiation of monocytes into phagocytic macrophages. The cholesterol sensitivity of the expression of ABCG1 is represented by the transcription factor pattern that is relevant for phagocytic differentiation (Example 7).

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**Examples****Example 1**5      **Identification of cholesterol loading and deloading candidate genes****Monocyte isolation and cell culture**

Monocytes were obtained from peripheral blood of healthy normolipidemic volunteers by leukapheresis and purified by counterflow elutriation. Purity of

10     isolated monocytes was >95% as revealed by FACS analysis. 10x10<sup>6</sup> monocytes were seeded into 100 mm<sup>2</sup> diameters cell culture dishes under serum free conditions in macrophage medium for 12 hours in a humidified 37°C incubator maintained with a 5% CO<sub>2</sub>, 95% air atmosphere. After 12 hours medium containing unattached cells was replaced by fresh macrophage medium supplemented with 50 ng/ml human  
15     recombinant M-CSF (this medium is the standard medium for any further incubations).

**Isolation of lipoproteins and preparation of AcLDL**

Lipoproteins were prepared from human plasma from healthy volunteer donors by  
20     standard sequential ultracentrifugation methods in a Beckman L-70 ultracentrifuge equipped with a 70 Ti rotor at 4°C to obtain LDL (d=1,006 to 1,063 g/ml) and HDL<sub>3</sub> (d=1,125 to 1,21 g/ml). All densities were adjusted with solid KBr. Lipoprotein fractions are extensively dialyzed with phosphate-buffered saline (PBS) containing 5 mM EDTA. The final dialysis step was in 0,15 mol/L NaCl in the absence of EDTA.  
25     Lipoproteins were made sterile by filtration through a 0.45 µm (pore-size) sterile filter (Sartorius).

LDL was acetylated by repeated addition of acetic anhydride followed by dialysis against PBS [19]. Modified LDL showed enhanced mobility on agarose gel electrophoresis.

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**Incubation of monocyte-macrophages with AcLDL and HDL<sub>3</sub>**

After 12 hours of preincubation cells were grown in the presence or absence (control) of 100 µg protein /ml AcLDL for further 3 day in medium. Then, the incubation medium was replaced with fresh medium and incubated with or without the addition of HDL<sub>3</sub> (100 µg/ml) for another 12 hours.

**Differential display**

Differential display screening was performed for new candidate genes that are regulated by cholesterol loading/deloading as described [20; 21]. In brief, 0,2 µg of total RNA isolated from monocytes at various incubations was reverse transcribed with specific anchored oligo-dT primers, using a commercially available kit (GeneAmp RNA PCR Core Kit, Perkin Elmer, Germany). The oligo-dT primers used had two additional nucleotides at their 3' end consisting of an invariable A at the second last position (3'-end) and A, C, G or T at the last position to allow a subset of mRNAs to be reverse transcribed. Here, a 13-mer oligo-dT (T101: 5'T11AG-2' ) was used in a 20-µl reaction at 2,5 µM concentration. One tenth of the cDNA was amplified in a 20-µl PCR reaction using the same oligo-dT and an arbitrary 10-mer upstream primer (D20 5'-GATCAATCGC-3'), 2,5 µM each, using 2,5 units of TAQ DNA Polymerase and 1.25 mM MgCl<sub>2</sub>. Amplification was for 40 cycles with denaturation at 94°C for 30 sec, annealing at 41°C for 1 min and elongation at 72°C for 30 sec with a 5 min extension at 72°C following the last cycle. All PCR reactions were carried out in a Perkin Elmer 9600 thermocycler (Perkin Elmer, Germany). PCR-products were separated on ready to use 10% polyacrylamide gels with a 5% stacking gel (CleanGel Large-10/40 ETC, Germany) under non-denaturating conditions using the Multiphor II electrophoresis apparatus (Pharmacia, Germany). The DNA fragments were visualized by silverstaining of the gel as previously described [22].

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**Cloning and sequencing of differentially expressed cDNAs**

cDNA bands of interest were cut out of the gel and DNA was isolated by boiling the gel slice for 10 min in 20 µl of water. A 4 µl aliquot was used for the following PCR-reaction in a 20µl volume. The cDNA was reamplified using the same primer set and PCR conditions as above, except, that the final dNTP concentration was 1mM each. Reamplified cDNAs were cloned in the pUC18-vector using ABCC8 (SUR)eClone-Kit (Pharmacia), sequenced on an automated fluorescence DNA sequencer using the AutoRead Sequencing Kit (Pharmacia, Germany) and used as probes for Northern blot analysis [23].

10

**Example 2****Northern Blot analyses of monocytes and macrophages after 3 days AcLDL incubation followed by 12 hours HDL<sub>3</sub> incubation**

Elutriated monocytes were incubated with AcLDL (100 µg/ml medium) for 2.5 days or differentiated for the same time without the addition of AcLDL as control. ABCG1 (ABC8) expression is 4 times stronger upregulated with AcLDL incubation than in differentiated monocytes .After the AcLDL incubation period cells were washed and incubated with HDL<sub>3</sub> for the next 12 hours or with medium alone as control. ABCG1 expression is almost completely downregulated by HDL3 incubation and only moderately decreased in control incubation as confirmed by Northern blot. For effective cholesterol loading monocytes must be differentiated to macrophage like cells. During this period scavenger receptors are upregulated and promote AcLDL uptake leading to cholesterol ester accumulation. To differentiated the cells prior to AcLDL-dependent cholesterol loading, we cultured the cells for four days in standard medium. At day four, cells were washed and incubated with AcLDL (100µg/ml medium) or in the absence of AcLDL as control for further one, two and three days to load the cells with cholesterol. At each timepoint cells were lysed with 0.1 % SDS and lipid was extracted as described in materials and methods and cellular cholesterol ester was determined by HPTLC-separation. Cells were loaded time

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dependently up to 120 nmol/mg cell protein after 3 days AcLDL loading, whereas in unloaded cells no cholestryl ester accumulation could be observed.

To distinguish HDL<sub>3</sub> dependent and independent cholesterol efflux cells were pulsed with AcLDL (100 µg/ml) for three days with the coincubation of <sup>14</sup>C-cholesterol (1.5 µCi/ml medium). Cells were washed and deloaded with HDL<sub>3</sub> (100 µg/ml) for 12 hours, 24 hours and 48 hours, respectively. Cells were incubated without the addition of exogenous lipid-acceptors as a control. After chase period the content of <sup>14</sup>C-cholesterol was determined in the medium and in the cells by liquid scintillation as described in material and methods. The efflux of cholesterol is expressed in percent of cellular DPMs of total DPMs (counts in the cells plus medium) With HDL<sub>3</sub>, the efflux is faster and more intense, than the efflux without the addition of HDL<sub>3</sub> as an endogenous lipid acceptor. After 12 hours cellular cholesterol content was reduced to 68 % with HDL<sub>3</sub>-dependent deloading, and 86 % in HDL<sub>3</sub>-independent deloading. After 48 hours only 35 % of loaded <sup>14</sup>C-cholesterol was observed in the cells treated with HDL<sub>3</sub>. In contrast, 70 % of loaded <sup>14</sup>C-cholesterol was found in untreated cells

In AcLDL pulsed cells the RNA-expression of ABCG1 is upregulated whereas no upregulation appears in the cells that were not loaded with AcLDL. Cells that were loaded for two days with AcLDL were deloaded with HDL<sub>3</sub> for 12, 24 and 48 hours (12h; 24h; 48h), and in the absence of exogenous lipid acceptors. The RNA-expression is downregulated again, in HDL<sub>3</sub> treated cells more intense than in cells treatet without any exogenous lipid acceptor.

25 **Materials:**

Macrophage medium (Macrophage-SFM) was obtained from Gibco Life Technologies, Germany. Human recombinant M-CSF was obtained from Genzyme Diagnostics, Germany, and antisense phosphorothioate oligonucleotides were supplied by Biognostics, Germany. All other chemicals were purchased from Sigma. Nylon membranes and a32P-dCTP were obtained from Amersham, Germany. <sup>14</sup>C-

cholesterol and 3H-choline chloride from NEN, Germany, and cell culture dishes are Becton Dickinson, Germany

#### **Isolation of total RNA and northern blotting**

5 Total RNA was isolated at each time-point, before and after AcLDL incubation, and after HDL<sub>3</sub> incubation, respectively. Washed cells were solubilized in guanidine isothiocyanate followed by sedimentation of the extract through cesium chloride [24]. For Northern analysis, 10 µg/lane of total RNA samples were fractionated by electrophoresis in 1,2% agarose agarose gel containing 6% formaldehyde and blotted onto nylon membranes (Schleicher & Schüll, Germany). After crosslinking with UV-irradiation (Stratalinker model 1800, Stratagene, USA), the membranes were hybridized with a cDNA probe for ABCG1 (ABC8). Hybridization and washing conditions were performed as recommended by the manufacturer of the membrane.

15 **Example 3**

#### **Westernblot analysis of monocytes and macrophages after cholesterol loading and deloading**

20 Protein expression of ABCG1 (ABC8) is upregulated in AcLDL-loaded and down-regulated in HDL<sub>3</sub>-deloaded monocyte-derived macrophages. Western blotting with a peptide antibody against ABCG1 as described in materials and methods is performed with 40 µg of total protein for each lane of SDS-PAGE. ABCG1-protein expression is shown in freshly isolated monocytes (day zero) and in differentiated monocytes (day four). From day four to day seven (5d; 6d; 7d) monocyte-derived macrophages were loaded with AcLDL or without AcLDL as control. AcLDL loaded cells from day 6 (6d) were deloaded with HDL<sub>3</sub> for 12, 24, and 48 hours and without exogenous added HDL lipid-acceptor. AcLDL increases the protein-expression, whereas HDL<sub>3</sub> decreases the expression to normal levels again.

**Protein isolation and determination**

At each timepoint cells were lysed with 0.1% SDS and the protein content was determined by the method of Lowry et al. [25].

5      **Generation of ABCG1 specific antibodies**

ABCG1 specific peptide antibodies were generated by immunization of chickens and rabbits with a synthetic peptide (Fa. Pineda, Berlin). The peptide sequence was chosen from the extracellular domain exIII amino acid residues 613-628 of ABCG1 comprising the amino acids REDLHCDIDETCHFQ (see sequence listing ID No. 10 53). After 58 days of immunization western blotting was performed with 1:1000 diluted serum and 1:10000 secondary peroxidase labelled antibody.

**Electrophoresis and immunoblotting**

SDS-polyacrylamide gelectrophoresis was performed with 40 $\mu$ g total cellular protein per lane. Proteins were transferred to Immobilon as reported. Transfer was confirmed by Coomassie Blue staining of the gel after the electroblot. After blocking for at least 2 hours in 5% nonfat dry milk the blot was washed 3 times for 15 minutes in PBS. Antiserum generated as described was used at 1:1000 dilution in 5% nonfat dry milk in PBS. The blot was incubated for 1 hour. After 4 times washing with PBS at room-temperature a secondary peroxidase-labelled rabbit anti chicken IgG-antibody (1:10000 diluted, Sigma) was incubated in 5% nonfat dry milk in PBS for 1 hour. After 2 times washing with PBS, detection of the immune complexes was carried out with the ECL Western blot detection system (Amersham International PLC, UK).

25

**Fluorescence resonance energy transfer:**

Monocytes were labelled with the specific antibodies for 15 minutes on ice, one antibody is labelled by biotin, the other one is labelled by phycoerythrin. After washing the cells were incubated with a Cy5-conjugated streptavidin for another 15 minutes.

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Distances between antibody labelled proteins on the cell surface is measured by energy transfer with a FACScan (Becton Dickinson). Following single laser excitation at 488 nm the Cy5 specific emmission represents an indirect excitation of Cy5 dependent on the proximity of the PE-conjugated antibody. The relative transfer efficiency was calculated following standardisation for the intensity of PE and Cy5 labelling and nonspecific overlap of fluorescence based on dual laser excitation and comparison to separately stained control samples.

#### Example 4

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##### Cholesterol sensitivity of ABCG1 (ABC8) and other members of the ABC-transporter family

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The influence of cholesterol loading and deloading on other members of the ABC-family was also investigated to find out the potential second half-size ABC transporter.

Further analysis has been performed to examine the expression pattern of all human ABC transporters in monocytes and monocyte derived macrophages as well as in cholesterol loaden and deloaden mononuclear phagocytes.

20

The experiments were performed by RT-PCR with cycle-variation to compare the expression in the quantitative part of the distinct PCR. Primer sets were generated from the published sequences of the ABC-transporters. A RT-PCR with GAPDH primers was used as control.

25

Several ABC-transporters are also cholesterol sensitive which further supports the function of ABC-transporters in cellular lipid trafficking (Table 2).

##### Semi-quantitative RT-PCR

30

All known ABC-transporters are tested for AcLDL/HDL<sub>3</sub> sensitive regulation of expression using RT-PCR with cycle-variation to compare the expression in the

quantitative part of the distinct PCR. 1 µg of total RNA was used in a 40 µl reverse transcription reaction, using the Reverse Transkription System (Promega, Corp. WI, USA). Aliquots of 5 µl of this RT-reaction was used in 50µl PCR reaction. After denaturing for 1,5 min at 94°C, 35 or less cycles of PCR were performed with 5 92,3°C for 44s, 60,8°C for 40s (standard annealing temperature differs in certain primer-combinations), 71,5°C for 46s followed by a final 5-min extension at 72°C. The Primer sets were generated from the published sequences of the ABC-transporters. A RT-PCR with primers specific for GAPDH was performed as control.

- 10 The expression pattern of ABC-transporters in monocytes, monocyte derived macrophages (3 days cultivated monocytes in serum free macrophage-SFM medium containing 50 ng/ml M-CSF), AcLDL incubated monocytes (3 days with 100 µg/ml) followed by HDL<sub>3</sub> (100 µg/ml) incubated monocytes is shown in Table 2. Expressed genes are tested for cholesterol sensitivity by semi-quantitative PCR.

15 **Example 5:**

**Functional analyses of the cholesterol sensitive ABCG1 (ABC8) transporter gene by antisense oligonucleotide experiments**

- 20 Antisense experiments were conducted in order to address the question, that beyond being regulated by cholesterol loading and deloading ABCG1 is directly involved in lipid loading and deloading processes.

In various experiments antisense oligonucleotides decreased the efflux of cholesterol and phosphatidylcholine to HDL<sub>3</sub>. During the loading period with AcLDL the cells were coincubated with 17 different antisense oligonucleotides. To measure the efflux of cholesterol and phospholipids the cells were pulsed in the loading period with 1,5 25 µCi/ml <sup>14</sup>C-cholesterol and 3µCi/ml <sup>3</sup>H-choline chloride. The medium was changed and during the chase period cells were incubated with or without HDL<sub>3</sub> for 12 hours. The <sup>14</sup>C-cholesterol and <sup>3</sup>H-choline content in the medium and in the cell lysate was measured and the efflux was determined in percent of total <sup>14</sup>C-cholesterol and <sup>3</sup>H-choline loading.

The most effective antisense oligonucleotide (AS Nr.2) inhibited cholesterol and phospholipids efflux relative to cells that were treated with control antisense (AS control). A dose dependent decrease in cholesterol efflux of 16,79% (5nmol AS) and 32,01% (10 nmol AS) could be shown, respectively.

5      **Antisense incubation**

To inhibit the induction of ABCG1 cells were treated with three different antisense oligonucleotides targeting ABCG1 or one scrambled control-antisense oligonucleotide during the AcLDL-incubation period.

10     **Determination of cholesterol and phosphatidylcholine efflux from monocytes in dependency of antisense oligonucleotide treatment**

To measure the efflux of cholesterol and phospholipids the cells were pulsed in addition to AcLDL-incubation with 1,5 µCi/ml <sup>14</sup>C-cholesterol and 3µCi/ml <sup>3</sup>H-choline chloride. The medium was changed and in chase period the cells were incubated with or without HDL<sub>3</sub> for 12 hours. Lipid extraction was performed according to the method of Bligh and Dyer [26]. The <sup>14</sup>C-cholesterol and <sup>3</sup>H-choline content in the medium and in the cell lysate was measured by liquid scintillation counting and the efflux was determined in percent of total <sup>14</sup>C-cholesterol and <sup>3</sup>H-choline loading as described [27]

15     **Computer analyses**

20     DNA and protein sequence analyses were conducted using programs provided by HUSAR, Heidelberg, Germany: <http://genius.embnet.dkfz-heidelberg.de:8080>.

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### Example 6

## Complete cDNA sequence of the human ATP binding cassette transporter 1 (ABCA1 (ABC1)) and assessing the cholesterol sensitive regulation of ABCA1 mRNA expression

## 5 cDNA Cloning and Primary Protein Structure

We have cloned a 6880-bp cDNA containing the complete coding region of the human ABCA1 gene (Figure 8). The open reading frame of 6603 bp encodes a 2201-amino acid protein with a predicted molecular weight of 220 kDa. This protein displays a 94% identity on the amino acid level in an alignment with mouse ABCA1 and can therefore be considered as the human ortholog.

## Tissue Distribution of ABCA1 mRNA Expression

In order to examine the tissue-specific expression of ABCA1 a multiple tissue RNA master blot containing poly A<sup>+</sup> RNA from 50 human tissues was carried out. Northern Blot analysis demonstrates the presence of a ABCA1 specific signal in all tissues. It is mostly prominent in adrenal gland, liver, lung, placenta and all fetal tissues examined so far (Table 1). The weakest signals are found in kidney, pancreas, pituitary gland, mammary gland and bone marrow.

## Sterol Regulation of ABCA1 mRNA Expression

In order to determine the regulation of ABCA1 in monocytes/macrophages during cholesterol loading/depletion Northern Blot analysis was performed. The cloned 1000-bp DNA fragment derived from PCR amplification of RNA from five day differentiated monocytes with primers ABCA1 3622f (*CGTCAGCACTCTGATGATGGCCTG-3'*) and ABCA1 4620r (*TCTCTGCTATCTCCAACCTCA-3'*) was hybridized to Northern Blots containing RNA of differentially cultivated monocytes (figure 12) As can be seen in lanes one to five, the ABCA1 mRNA is increased during in vitro differentiation of freshly isolated monocytes until day five. Longer cultivation results in a total loss of

expression. When the cells were incubated in the presence of AcLDL to induce sterol loading (lanes 6-8) beginning at day four, a much stronger accumulation of mRNA can be detected in comparison to control cells (lanes 2-5). When these cells were cultured with HDL<sub>3</sub> as cholesterol acceptor for 12h, 24h and 48h (lanes 9-11) the ABCA1 signal significantly decreases with respect to control cells incubated in the absence of HDL<sub>3</sub> (lanes 12-14). Taken together, these results indicate that ABCA1 is a sterol-sensitive gene which is induced by cholesterol loading and downregulated by cholesterol depletion.

Cell culture.

Peripheral blood monocytes were isolated by leukapheresis and counterflow elutriation (19JBC). To obtain fractions containing >90% CD 14 positive mononuclear phagocytes, cells were pooled and cultured on plastic Petri dishes in macrophage SFM medium (Gibco BRL) containing 25 U/ml recombinant human M-CSF (Genzyme) for various times in 5% CO<sub>2</sub> in air at 37°C. The cells were incubated in the absence (differentiation control) or presence of AcLDL (100 µg/ml) to induce sterol loading. Following this incubation the cells were cultured in fresh medium supplemented with or without HDL<sub>3</sub> (100 µg/ml) for additional times in order to achieve cholesterol efflux from the cells to its acceptor HDL<sub>3</sub>.

Preparation of RNA and Northern blot analysis.

Total cellular RNA was isolated from the cells by guanidium isothiocyanate lysis and CsCl centrifugation (Chirgwin). The RNA isolated was quantitated spectrophotometrically and 15 µg samples were separated on a 1.2% agarose-formaldehyde gel and transferred to a nylon membrane (Schleicher & Schüll). After crosslinking with UV-irradiation (Stratalinker model 1800, Stratagene), the membranes were hybridized with a 1000 bp DNA fragment derived from PCR amplification with primers ABCA1 3622f and ABCA1 4620r, stripped and subsequently hybridized with a human β-actin probe. In order to determine the tissue-specific expression of ABCA1 a multiple tissue RNA master blot containing

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poly A<sup>+</sup> RNA from 50 human tissues was purchased from Clontech. The probes were radiolabeled with [ $\gamma$ -<sup>32</sup>P]dCTP (Amersham) using the Oligolabeling kit from Pharmacia. Hybridization and washing conditions were performed following the method described previously (Virca).

5      cDNA cloning of human ABCA1

Based on sequence information of mouse ABCA1 cDNA we designed primers for RT-PCR analysis in order to amplify the human ABCA1 (ABC1) cDNA. Approximately 1 $\mu$ g of RNA from five day differentiated mononuclear phagocytes was reverse transcribed in a 20  $\mu$ l reaction using the RNA PCR Core Kit from Perkin 10 Elmer. An aliquot of the cDNA was used in a 100  $\mu$ l PCR reaction performed with AmpliTaq Gold (Perkin Elmer) and the following primer combinations: (primer names indicate the position in the corresponding mouse cDNA sequence):

15      *mABC1-144f* (5'-CAAACATGTCAGCTGTTACTGGA-3') and

15      *mABC1-643r* (5'-TAGCCTTGCAAA-AATACCTCTG-3'),

20      *mABC1-1221f* (5'-GTTGGAAAGATTCTCTATACACACCTG-3') and

20      *mABC1-1910r* (5'-CGTCAGCACTCTGATGATGGCCTG-3'),

25      *mABC1-3622f* (5'-TCTCTGCTATCTCCAACCTCA-3') and

25      *mABC1-4620r* (5'-ACGTCTTCACCAGGTAATCTGAA-3'),

30      *mABC1-5056f* (5'-CTATCTGTCATCTTGCGATG-3') and

30      *mABC1-5857r* (5'-CGCTTCCTCCTATAGATCTTGGT-3'),

35      *mABC1-6093f* (5'-AAGAGAGCATGTGGA-GTTCTTG-3') and

35      *mABC1-7051r* (5'-CCCTGTAATGGAATTGTGTTCTC-3'),

40      *hABC1-540f* (5'-AACCTCTCTGGGTTCTGTATC-3') and

40      *hABC1-1300r* (5'-AGTTCTGGAA-GGTCTTGTTCAC-3'),

45      *hABC1-1831f* (5'-GCTGACCCCTTGAGGACATGCG-3') and

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*hABC1-3701r (5'-ATAGGTCAGCTCATGCCCTATGT-3'),*

*hABC1-4532f (5'-GCTGCC-TCCCTCCACAAAGAAAAC-3') and*

*hABC1-5134r (5'-GCTTGCTGACCCGCTCC-TGGATC-3').*

*hABC1-5800f (5'-GAGGCCAGAACATGACATCTTAGAA-3') and*

*hABC1-6259r (5'-CTTGACAACACTTAGGGCACAAAT-3').*

5

*All PCR products were cloned into the pUC18 plasmid vector and the nucleotide sequences were determined on a Pharmacia ALFexpress sequencer using the dideoxy chain-termination method and fluorescent dye-labeled primers.*

10

#### Example 7

##### *Identification of the 5'end of ABCG1*

We could partially prove the 5'-end of ABCG1 published by Chen [7] that differs from the 5'-end published by Croop [6] obtained from the mRNA of human

15

monocytes/macrophages using a 5' RACE approach. In detail the sequence according to Chen et al. downstream of position 25 was in agreement with our own data. In contrast, our identified sequence differs from the one reported by Chen [7] and Croop [6] at a site upstream of position 25 (Chen [7]). The sequence SEQ ID NO: 32 shows the newly identified 5'-end followed by the sequence published by Chen [7] from position 25.

20

##### *Molecular cloning and characterisation of the ABCG1 5'UTR*

We identified several fragments by screening of a  $\lambda$  phage library which contained a total of app. 3 kb of the 5' UTR upstream sequence of the human ABCG1 gene. The

25

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sequence that comprises the 5'UTR and part of exon 1 (described above) are given in SEQ ID NO: 54.

The promoter activity of this sequence was proven by luciferase reporter gene assays in transiently transfected CHO cells.

- 5 Putative transcription factor binding sites within the promoter region with the highest likelihood ratio for the matched sequence as deduced from the TransFac database, GFB, Braunschweig, Germany. Multiple binding sites for SP-1, AP-1, AP-2 and CCAAT-binding factor (C/EBP family) are present within the first 1 kb of the putative promoter region.
- 10 Additionally, a transcription factor binding site involved in the regulation of apolipoprotein B was identified.

**Example 8**

- 15 **Characterization of the human ABCA8 full length cDNA**  
The putative ABCA8 coding sequence is app. 6.5 kb in size. We successfully cloned and sequenced a 1kb segment of the human ABCA8 cDNA that encodes the putative second nucleotide binding site of the mature polypeptide (the sequence is shown in the sequence listing). The nucleotide sequence exhibits a 73% homology with the known human ABCA1 (ABC1) cDNA sequence.

- 25 We identified an alternative transcript in the cloned 1 kb coding region which consists of a 72 bp segment (see sequence listing). Genomic analysis of this region revealed that the alternative sequence is identical with a complete intron suggesting that the alternative mRNA is generated by intron retention. The retained intron introduces a preterminal stop codon and thus may code for a truncated ABCA8 variant.

ABCA8 also shows a cholesterol sensitive regulation of the mRNA expression (Table 2).

5 Tissue expression of ABCA8 is shown in table 1.

#### Example 9

##### Characterisation of the regulation of ABC transporter during differentiation of keratinocytic cells (HaCaT)

Differentiation of epidermal keratinocytes is accompanied by the synthesis of specific lipids composed mainly of sphingolipids (SL), free fatty acids (FFA), cholesterol (CH), and cholesterol sulfate, all involved in the establishment of the epidermal permeability barrier. The skin and, in particular, the proliferating layer of the epidermis is one of the most active sites of lipid synthesis in the entire organism. Cholesterol synthesis in normal human epidermis is LDL-independent, and circulating cholesterol levels do not affect the cutaneous de novo cholesterol synthesis. Fully differentiated normal human keratinocytes lack LDL receptors or its expression is very low, whereas in the normal human epidermis only basal cells express LDL receptors.

During keratinocyte differentiation a shift from polar glycerophospholipids to neutral lipids (FFA, TG) and also a replacement of short chain FFA by long chain highly saturated FFA is observed. The most important lipids for the barrier function of the skin are sphingolipids that account for one third of the lipids in the cornified layer, and consist of a large ceramide fraction as a result of glucosylceramide degradation by intercellular glycosidases and de novo synthesis of ceramide .

Glucosylceramide is synthesized intracellularly and stored in lamellar bodies and glucosylceramide synthase expression was found up-regulated during the differentiation of human keratinocytes.

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Cholesterol sulfate is formed by the action of cholesterol sulfotransferase during keratinocyte differentiation . Cholesterol sulfate and the degrading enzyme steroid sulfatase are present in all viable epidermal layers, with the highest levels in the stratum granulosum. The gradient of cholesterol sulfate content across the stratum corneum (from inner to outer layers), and progressive desulfation of cholesterol sulfate regulate cell cohesiveness and normal stratum corneum keratinization and desquamation, respectively. Cholesterol sulfate induces transglutaminase 1 and the coordinate regulation of both factors is essential for normal keratinization .

- 10 The final step in lipid barrier formation involves lamellar body secretion and the subsequent post-secretory processing of polar lipids into their nonpolar lipid products through the action of hydrolytic enzymes that are simultaneously released ( $\beta$ -glucocerebrosidase, phospholipases, steroid sulfatase, acid sphingomyelinase).
- 15 Disruption of the permeability barrier results in an increased cholesterol, fatty acid, and ceramide synthesis in the underlying epidermis. It has been shown that mRNA levels for the key enzymes required for cholesterol, fatty acid, and ceramide synthesis increased rapidly after artificial barrier disruption .
- 20 Currently the lipid transport systems in keratinocytes are poorly characterized. Several fatty acid transport related proteins have been identified in keratinocytes: plasma membrane fatty acid transport proteins (FATPs) and intracellular fatty acid binding proteins (FABPs), most of them exhibiting high affinity for essential fatty acids. The expression of epidermal FABPs is up-regulated in hyperproliferative and inflammatory skin diseases, during keratinocyte differentiation and barrier disruption
- 25

30 Based on our data on macrophages, we propose several ABC transporters as putative candidates for cellular lipid export in keratinocytes. We have examined the expression of all known ABC transporters during HaCaT cells differentiation. The human HaCaT cell line has a full epidermal differentiation capacity. Keratinocytes grown in

vitro as a monolayer at low calcium concentration (< 0.1 mM) can be differentiated by increasing calcium concentration in the culture medium (1-2 mM). We cultured HaCaT cells as a monolayer in calcium-free RMPI (Gibco) medium mixed with standard Ham's F12 medium at a ratio 3:1 supplemented with 10% chelex-treated  
5 FCS, Penicillin and Streptomycin. The final concentration of calcium in above medium was 0.06 mM. When the cells reached confluence (usually on 5<sup>th</sup> day of the culture), calcium concentration was enhanced up to the level of 1.2 mM. The cells were seeded at a density of  $2 \times 10^5 / \text{cm}^2$  in 60 mm culture dishes. The culture medium was replaced every two day and the cells were harvested after 24 h, 48 h, 4 d, 6 da,  
10 8 d and 10 d in culture, respectively. Total RNA from HaCaT cells was isolated using the isothiocyanate/cesium chloride-ultracentrifugation method.

The expression of all known human ABC transporters was examined during HaCaT cell differentiation (24 h, 48 h, 4 d, 6 d, 8 d, 10d, respectively) using a semi-quantitative RT-PCR approach (Table 6). The primer sets were generated from the published sequences of the ABC-transporters. Primers specific for GAPDH were used as a control. As a marker of keratinocyte differentiation CGT (ceramide glucosyl transferase) gene expression was assessed. Three of the transporters examined, ABCB1 (MDR1), ABCB4 (MDR3), ABCD3 (PMP70), were not expressed.  
15 ABCC6 (MRP6), ABCA1 (ABC1),ABCD2 (ALDR and ABCB9 (est122234) were expressed at low levels (Table 6)

Most of the other transporters exhibited a biphasic expression pattern or were downregulated during keratinocyte differentiation. There was, however, a high expression of ABCG1 (ABC8), ABCA8 (new) and ABCC3 (MRP3) indicative for their involvement in terminal keratinocyte lipid secretion for cholesterol, FFAs and ceramide-backbone lipids.. The two peroxisomal ABC transporters, ABCD2 (ALDR) and ABCD1 (ALDP) that mediate the transport of very long chain fatty acids into peroxisomes were initially expressed at relatively low levels and subsequently downregulated during differentiation. This is in agreement with the replacement of  
25  
30

short chain fatty acids by very long chain fatty acids during keratinocyte differentiation.

**Example 10:**

- 5 Sequencing of ABCA1 cDNA and genomic structure in five families of patients with Tangier disease revealed different mutations in the ABCA1 gene locus. These patients have different mutations at different positions in the ABCA1 gene, that result in changes in the protein structure of ABCA1. Family members that are heterozygous for these mutations show lowered levels of serum HDL, whereas the  
10 homocysteine patients have extremely reduced HDL serum levels.

Claims:

1. A polynucleotide comprising a member selected from the group consisting of:

- 5           (a) a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:2;
- (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
- (c) a polynucleotide fragment of the polynucleotide of (a) or (b).

10          2. The polynucleotide of claim 1 wherein the polynucleotide is DNA.

15          3. A vector containing one or more of the polynucleotides of claim 1 and 2.

15          4. A host cell containing the vector of claim 3.

20          5. A process for producing a polypeptide comprising: expressing from the host cell of claim 4 the polypeptide encoded by said DNA.

20          6. A polypeptide selected from the group consisting of

- 25           (a) a polypeptide having the deduced amino acid sequence of SEQ ID NO:2 and fragments, analogs and derivatives thereof, and
- (b) a polypeptide comprising amino acid 1 to amino acid 2201 of SEQ ID NO:2.

25          7. An antibody capable to bind to the polypeptide of claim 6.

30          8. A diagnostic kit for the detection of the polypeptide of claim 6.

9. Use of a polypeptides encoded by a polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide as set forth in SEQ ID NO:1, 3, 4 and 6 to 31;

- (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
- (c) a polynucleotide fragment of the polynucleotide of (a) or (b)

in an assay for detecting modulators of said polypeptides.

10. Modulator of a polypeptides encoded by a polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide as set forth in SEQ ID NO:1, 3, 4 and 6 to 31;

- (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
- (d) a polynucleotide fragment of the polynucleotide of (a) or (b)

11. A pharmaceutical comprising the modulator of claim 10

12. An assay for detecting polypeptides encoded by a polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide as set forth in SEQ ID NO:1, 3, 4 and 6 to 32 and 54;

(b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and

(c) a polynucleotide fragment of the polynucleotide of (a) or (b)

Figure 1

2588 GA TCAATCGCAT TCATTTAAG AAATTATACC TTTTAGTAC TTGCTGAAGA  
 2641 ATGATTCAGG GTAAATCACA TACTTTGTT AGAGAGCGA GGGGTTAAC CCGAGTCACC  
 2701 CAGCTGGCT CATACATAGA CAGCAGCTGT GAAGGATTGA ATGCAGGTT CAGGTGGAGG  
 2761 GAACACGTCG ACACCATCTC CACTGAGCCA TGCAAGACATT TTTAAAGCT ATACACAAAAA  
 2821 TTGTGAGAAG ACATGGCCA ACTCTTCAA AGTCTTCTT TTCCACGTG CTTCTTATT  
 2881 TAACGAAAT ATATTGTTG TTCTTCTTA AAAAAAAA 2890

Figure 2

1 CAACACATGTCAGCTGTTACTGGAAGTGGCCTGGCCTCTATTATCTCCGTATGCCAACCCCC 60  
 61 TCTGTTCCGGCTGAGCTACCCACCCATGAACAACTGAATGCCATTCCAAATAAGGCC 120  
 121 ATGCCCTCTGCAGGAACACTTCCCTGGGTTCAAGGGGATTATCTGTAATGCCAACCCCC 180  
 1 M P S A G T L P W V Q G I I C N A N N P 20  
 181 TGTTTCCGTTACCCGACTCTGGGAGGCTCCCGGAGTGTGGAAACTTAAACAAATCC 240  
 21 C F R Y P T P G E A P G V V G N F N K S 40  
 241 ATTGTGGCTGCCCTGTTCTCAGATGCTGGAGGCTCTTTATACAGCCAGAAAGACACC 300  
 41 I V A R L F S D A R R L L L Y S Q K D T 60  
 301 AGCATGAAGGACATGCCAAAGTCTGAGAACATTACAGCAGATCAAGAAATCCAGCTCA 360  
 61 S M K D M R K V L R T L Q Q I K K S S S 80  
 361 AACCTGAAGGCTTACAGATTCCTGGTGGACATGAAACCTTCTCTGGGTTCCGTATCAC 420  
 81 N L K L Q D F L V D N E T F S G F L Y H 100  
 421 AACCTCTCTCTCCCAAAGTCTACTGTGGACAAGATGCTGAGGGCTGATGTCATTCTCC 480  
 101 N L S L P K S T V D K M L R A D V I L H 120  
 481 AAGGTATTTTCAAGGCTACCAAGTTACATTTGACAAGTGTGTCAATGGATCAAATCA 540  
 121 K V F L Q G Y Q L H L T S L C N G S K S 140  
 541 GAAGAGATGATTCAACTTGGTGACCAAGAAGTCTGAGCTTTGTCAGGCTACCAAGGGAG 600  
 141 E E M I Q L G D Q E V S E L C G L P R E 160  
 601 AAACCTGGCTGCAGCAGCGAGTACTCGTTCCAACATGGACATCCTGAAGCCAATCTG 660  
 161 K L A A A E R V L R S N M D I L K P I L 180  
 661 AGAACACTAAACTCTACATCTCCCTTCCCAGCAAGGAGCTGGCCGAAGGCCACAAAACA 720  
 181 R T L N S T S P F P S K E L A E A T K T 200  
 721 TTGCTGCATAGCTTGGGACTCTGGCCAGGAGCTTCAGCATGAGAAGCTGGAGTGAC 780  
 201 L L H S L G T L A Q E L F S M R S W S D 220  
 781 ATCGCACAGGAGGTGATGTTCTGACCAATGTGACACAGCTCCAGCTCCACCCAAATC 840  
 221 M R Q E V M F L T N V N S S S S S T Q I 240  
 841 TACCAAGGCTGTGCTCGTATTGCTGCAGGAGCTTCAGCATGAGAAGCTGGAGTGAC 900  
 241 Y Q A V S R I V C G H P E G G G L K I K 260  
 901 TCTCTCAACTGGTATGAGGACACAAACTACAAAGCCCTCTTGGAGGCAATGGCACTGAG 960  
 261 S L N W Y E D N N Y K A L F G G N G T E 280

961 GAGATGCTAAACCTCTATGACAACCTACAACCTCCTTACTGCAATGATTGATGAG 1020  
 281 E D A E T F Y D N S T T P Y C N D L M K 300  
 1021 AATTGGAGTCAGTCCTCTTCCGCATTATCTGGAAAGCTCTGAAGCCGCTGCTCGTT 1080  
 301 N L E S S P L S R I I W K A L K P L L V 320  
 1081 GGGAGATCCTGTATAACACTGACACTCCAGCCAAGGCAGGTATGGCTGAGGTAAAC 1140  
 321 G K I L Y T P D T P A T R Q V M A E V N 340  
 1141 AACACCTCCAGGAACTGGCTGTGTTCCATGATCTGAGGCATGGGAGGAACCTCAC 1200  
 341 K T F Q E L A V F H D L E G M W E E L S 360  
 1201 CCAAGATCTGGACCTTCATGGAGAACAGCCAAGGAATGGACCTTGTCCGGATGCTGTT 1260  
 361 P K I W T F M E N S Q E M D L V R M L L 380  
 1261 GACAGCAGGGACAATGACCACTTTGGGAAACAGCAGTTGGATGGCTAGATTGGACAGCC 1320  
 381 D S R D N D H F W E Q Q L D G L D W T A 400  
 1321 CAAGACATCGTGGCGTTTGGCCAAGCACCCAGGAGTGTCCAGTCAGTAAATGGTCT 1380  
 401 Q D I V A F P L A K H P E D V Q S S N G S 420  
 1381 GTGTACACCTGGAGAGAAGCTTCACCGAGACTAACAGGCAATCCGGACCATACTCGC 1440  
 421 V Y T W R E A F N E T N Q A I R T I S R 440  
 1441 TTCATGGAGTCGTCAACCTGAAAGCTAGAACCCATAGCAACAGAACACTGGCTCATC 1500  
 441 F M E C V N L N K L E P I A T E V V W L I 460  
 1501 ACAAGTCCATGGAGCTGCTGGATGAGAGGAATCTGGGCTGGTATTGTGTTACTGGA 1560  
 461 N K S M E L L D E R K F W A G I V F T G 480  
 1561 ATTACTCCAGGCAGCATGGCTGCCCCATCATGTCAGTACAAGATCCGAATGGACATT 1620  
 481 I T P G S I E L P H H V K Y K I R M D I 500  
 1621 GACAATGTGGAGAGGACAAAATCAAGGATGGTACTGGGACCTGGTCTCGAGCT 1680  
 501 D N V E R T N K I K D G Y W D P G P R A 520  
 1681 GACCCCTTGAGGCCATGGGTACGTCGGGGGGCTTCGCTACTTGCAAGGATGTGTTG 1740  
 521 D P F E D M R Y V W G G F A Y L Q D V V 540  
 1741 GAGCAGGAATCATCAGGTGCTGACGGGACCCGAGAAGAAAATGGTGTATATGCAA 1800  
 541 E Q A I I R V L T G T E K K T G V Y M Q 560  
 1801 CAGATGCCCTATCCCTGTTACGGTGTGACATCTTCGCGGGTGTGAGCCGGTCATG 1860  
 561 Q M P Y P C Y V D D I F L R V M S R S M 580  
 1861 CCCCTTCATGACGCTGGCTGGATTTACTCAGTGGCTGTGATCATCAAGGGCATGTC 1920  
 581 P L F M T L A W I Y S V A V I I K G I V 600  
 1921 TATGAGAAGGGCAGGGCTGAAAGAGACATGGGATCATGGGCTGGACACAGCATC 1980  
 601 Y E K E A R L K E T M R I M G L D N S I 620  
 1981 CTCTGGTTAGCTGGTTCAATTAGTAGGCTCATTCCTCTTGTGAGCGCTGGCTGCTA 2040  
 621 L W F S W F I S S L I P L L V S A G L L 640  
 2041 GTGGTCATCCTGAAAGTTAGGAAACCTGCTGCCCTACAGTGATCCCAGCGTGGTGTGTC 2100  
 641 V V I L K L G N L L P Y S D P S V V F V 660  
 2101 TTCCCTGTCCGTGTTGCTGTGGTGACAATCCTGCAGTGCTTCCCTGATTAGCACACTCTTC 2160

661 F L S V F A V V T I L Q C F L I S T L F 680  
 2161 TCCAGAGCCAACCTGGCAGCGCTGTGGGGCATCATCTACTTCACGCTGTACCTGCC 2220  
 681 S R A N L A A A C G G I I Y F T L Y L P 700  
 2221 TAGCTCTGTGTGGCATGGCAGGACTACGTGGCTTCACACTCAAGATCTCGCTAGC 2280  
 701 Y V L C V A W Q D Y V G F T L K I F A S 720  
 2281 CTGCTGTCTCTGTGGCTTTGGGTTGGCTGTGAGTACTTGCCCTTTTGAGGAGCAG 2340  
 721 L L S P V A F G F G C E Y F A L F E E Q 740  
 2341 GGCGATTGGAGTGCAGTGGGACAACCTGTTGAGACTCCGTGGAGGAAGATGGCTTAAT 2400  
 741 G I G V Q W D N L F E S P V E E D G F N 760  
 2401 CTCACCACCTCGGTCTCCATGATGCTGTTGACACCTTCCTCTATGGGTGATGACCTGG 2460  
 761 L T T S V S M M L F D T F L Y G V M T W 780  
 2461 TACATTGAGGTGCTCTTCCAGGGCAGTACCGAAATCCGAGGCCCTGGTATTTCCCTTG 2520  
 781 Y I E A V F P G Q Y G I P R P W Y F P C 800  
 2521 ACCAAGTCTACTGGTTGGCAGGAAAGTGTGAGAAGAGGCCACCTGGTCCAACAG 2580  
 801 T K S Y W F G E E S D E K S H P G S N Q 820  
 2581 AAGAGAAATATCGAAATCTGCATGGAGGAGGAACCCACCCACTGAAAGCTGGCGTGTCC 2640  
 821 K R I S E I C M E E E P T H L K L G V S 840  
 2641 ATTCAAGAACCTGGTAAAAGTCTACCGAGATGGGATGAAGGTGGCTGTGATGGCTGGCA 2700  
 841 I Q N L V K V Y R D G M K V A V D G L A 860  
 2701 CTGAATTTTATGAGGGCCAGATCACCTCTTCTGGCCACAATGGAGCGGGGAAGACG 2760  
 861 L N F Y E G Q I T S F L G H N G A G K T 880  
 2761 ACCACCATGTCAATCTGACCGGGTTGTCACCCCCCGACCTCGGGCACCGCTACATCTG 2820  
 881 T T M S I L T G L F F P T S G T A Y I L 900  
 2821 GGAAAGACATTGCTCTGAGATGAGCACCATTGGCAGAACCTGGGGTCTGTCCCCAG 2880  
 901 G K D I R S E M S T I R Q N L G V C P Q 920  
 2881 CATAACGTGCTGTTGACATGCTGACTGTGAGAACACATCTGGTTCTATGCCCGCTTG 2940  
 921 H N V L F D M L T V E E H I W F Y A R 940  
 2941 AAAGGGCTCTGTGAGAACGACGTGAAGGGAGATGGAGCAGATGCCCTGGATGTGCT 3000  
 941 K G L S E K H V K A E M E Q M A L D V G 960  
 3001 TTGCCATCAAGCAAGCTGAAAACCAAACAGCCAGCTGTGAGCTGAATGAGAGAAAG 3060  
 961 L P S S K L K S K T S Q L S G G M Q R K 980  
 3061 CTATCTGTGGCTTGGCTTGTGGGGATCTAAGGTGTCACTTCTGGATGAACCCACA 3120  
 981 L S V A L A F V G G S K V V I L D E P T 1000  
 3121 GCTGGTGTGGACCCCTACTCCCGCAGGGAAATGGGAGCTGCTCTGAAATACCGACAA 3180  
 1001 A G V D P Y S R R G I W E L L L K Y R Q 1020  
 3181 GGCGGCACCATATTCTCTCTACACACCATGGATGAAGCGGAGCTGTGGGGACAGG 3240  
 1021 G R T I I L S T H H M D E A D V L G D R 1040  
 3241 ATTGGCATCATCTCCCAGGGAGCTGCTGTGGCTCTCCCTGTTCTGAAGAAC 3300  
 1041 I A I I S H G K L C C V G S S L F L K N 1060  
 3301 CAGCTGGAACAGGCTACTACCTGACCTTGGTCAAGAAAGATGTGGAATCCTCCCTCAGT 3360

1061 Q L G T G Y Y L T L V K K D V E S S L S 1080  
 3361 TCC TGC AGAA ACAGT AGTAG CACT GTG TCAT ACC TGA AAA AGG AGG ACG ATG TTT CTCA G 3420  
 1081 S C R N S S S T V S Y L K K E D S V S Q 1100  
 3421 ACC AGT TCT GAT CTG TGG CAG CG ACC ATG AGA GTG ACAC CCG TGA CC ATG ATC GTC 3480  
 1101 S S S D A G L G S D H E S D T L T I D V 1120  
 3481 TCT GCT ATC TCC AAC CT CAT CAG GA AGG ATG TG CTG TA AGG CCG GCT GG TG AA GACA TA 3540  
 1121 S A I S N L I R K H V S E A R L V E D I 1140  
 3541 GGG CAT GAG CTG AGC CT ATG TG CTG CC AT ATG AAG CTG TA AGG AGG GAG CCT TTG GAA 3600  
 1141 G H E L T Y V L P Y E A A K E G A F V E 1160  
 3601 CTCTT TCAT GAG ATT GAT GAC CGG CT TCAG ACAC TGG GAT TT CTA GTT ATG GC AT CTCA 3660  
 1161 L F H E I D D R L S D L G I S S Y G I S 1180  
 3661 GAG AC GAC CCT GG AA GAA AT TCC TCA AGG TGG CGA AGA GAG TGG GGT GG AT GCT GAG 3720  
 1181 E T T L E E I F L K V A E E S G V D A E 1200  
 3721 AC CT CAG AT GG TA CCT TGG CAG CA AGA AAC AGG CCG GCT TCC GG GAC AAG CAG AGC 3780  
 1201 T S D G T L P A R R N R R A F G D K Q S 1220  
 3781 TGT CTG CCG CGT TC ACT GAG AT GAT GCT GTC AT GCA AA AT GATT CTG AC AT AGAC CCA 3840  
 1221 C L R P F T E D D A A D P N D S D I D P 1240  
 3841 GA AT CC CAG AGA GAG ACAG ACT TG CT CAG TGG GAT GG AT GG CA AAG GG CT TAC CAG GTG AAA 3900  
 1241 E S R E T D L L S G M D G K G S Y Q V K 1260  
 3901 GG CT GG AA ACT TA CAC AG CA AC AG TGT GG CC CTT TGT GG AA AGA GACT GCT AAT GG C 3960  
 1261 G W K L T Q Q Q F V A L L W K R L L I A 1280  
 3961 AG AC GG ACT CGG AA AGG AT TTT TG CT CAG AT TG TCT GG CAG TGT GT TGT CTG CATT 4020  
 1281 R R S R K G F F A Q I V L P A V F V C I 1300  
 4021 G C C C T T G T G T C A G C C T G A T G T G C C A C C C T T G G C A A G T A C C C C A G C C T G G A A C T C A G 4080  
 1301 A L V F S L I V P P F G K Y P S L E L Q 1320  
 4081 C C T G G A T G T A C A C G A A C A G T A C A C T T G T C A G G C A A T G A T G T C C T G A G G A C A C G G G A 4140  
 1321 P W M Y N E Q Y T F V S N D A P E D T G 1340  
 4141 A C C C T G G A A C T T A A C G C C C T C A C C A A A G A C C T G G C T C G G G A C C C G T G T A T G G A 4200  
 1341 T L E L L N A L T K D P G F G T R C M E 1360  
 4201 G G G A A C C A A T C C C A G A C C G C C T G C C A G G C A C C G G A G G A A G A C T G G A C C A C T G C C C C A 4260  
 1361 G N P I P D T P C Q A G E E E W T T A P 1380  
 4261 G T T C C C C A G A C C A T C A T G G A C C T T C C A G A A T G G A A C T G G A C A A T G C A G A A C C C T C A 4320  
 1381 V P Q T I M D L F Q N G N W T M Q N P S 1400  
 4321 C C T G C A T G C C A G G T G A C G C G A C A A A T C A A G A A G A T G C T G C C T G T G T C C C C A G G G 4380  
 1401 P A C Q C S S D K I K K M L P V C P P G 1420  
 4381 G C A G G G G G G C T G C C T C C T C C A A A G A A A C A A A C T G C A G A T A T C C T C A G G A C C T G 4440  
 1421 A G G L P P P Q R K Q N T A D I L Q D L 1440  
 4441 A C A G G A A G A A A C A T T C G G A T T A C T G G T G A A G A C T A T G T G C A G A T C A T A G C C C A A A G C 4500  
 1441 T G R N I S D Y L V K T Y V Q I I A K S 1460  
 4501 T T A A A G A A C A A G A T C T G G G T G A A T G A G T T A C G T A T G G C G G T T T C C C T G G G T G C A G T 4560

1461 L K N K I W V N E F R Y G G F S L G V S 1480  
 4561 AATACTCAAGCACCTCCTCGAGTCAGAAGTAAATGATGCCACAAACAAATGAAGAAA 4620  
 1481 N T Q A L P P S Q E V N D A T K Q M K K 1500  
 4621 CACCTAAAGCTGGCCAGGCACAGTCTGCAGATCGATTCTCAACAGCTTGGGAAGATT 4680  
 1501 H L K L A K D S S A D R F L N S L G R F 1520  
 4681 ATGACAGGACTGGACACCAGAAAATATGTCAGGTGTTCAATACAAGGGCTGGCAT 4770  
 1521 M T G L D T R N N V K V W F N N K G W H 1540  
 4741 GCAATCAGCTTTCCTGAAATGTCATCAACATGCCATTCTCCGGGCAACCTGCAAAG 4800  
 1541 A I S S F L N V I N N A I L R A N L Q K 1560  
 4801 GGAGAGAACCTCTAGCCATTATGGAATTACTGCITTCATCATCCCCCTGAATCTCACCAG 4860  
 1561 G E N P S H Y G I T A F N H P L N L T K 1580  
 4861 CAGCACCTCTCAGAGGTGGCTCCGATGACCAACTCATGGATGTGCTTGTGTCATCTGT 4920  
 1581 Q Q L S E V A P M T T S V D V L V S I C 1600  
 4921 GTCATCTTGCATGTCTCGTCCCAGCCAGCTTGTCGTATTCTGATCCAGGAGCGG 4980  
 1601 V I F A M S F V P A S F V V F L I Q E R 1620  
 4981 GTCAAGAACCAAACACCTGCACTTCATCATGGAGTGAAGCCTGTCATCTACTGGCTC 5040  
 1621 V S K A K H L Q F I S G V K P V I Y W L 1640  
 5041 TCTAATTGTCGGATATGTCGAATTACGTTGTCCTGCCACACTGGTCATTATCATC 5100  
 1641 S N F V W D M C N Y V V P A T L V I I I 1660  
 5101 TTCACTGCTTCAGCAGAAGTCATATGTCCTCCACCAATCTGCCGTGCTAGCCCTT 5160  
 1661 F I C F Q Q K S Y V S S T N L P V L A L 1680  
 5161 CTACTTTGCTGTATGGTGGTCATCACACCTCTCATGTAACCCAGCCTCTTGTTGTC 5220  
 1681 L L L L Y G W S I T P L M Y P A S F V F 1700  
 5221 AAGATCCCCACACAGCTATGTGGTGTCAACAGCGTGAACCTCTTCAATTGGCATTAAAT 5280  
 1701 K I P S T A Y V V V L T S V N L F I G I N 1720  
 5281 GGCGAGCGTGGCCACCTTGTCTGGAGCTGTCACCGACAATAAGCTGAATAATATCAAT 5340  
 1721 G S V A T F V L E L F T D N K L N N I N 1740  
 5341 GATATCCTGAAGTCCTGTCATTCACCTCCACATTGGCTGGAGCAGGGCTCATC 5400  
 1741 D I L K S V F L I F P H F C L G R G L I 1760  
 5401 GACATGGTAAAAACCAAGCCAATGGCTGATGCCCTGGAAAGGTTGGGGAGAACATCGCTT 5460  
 1761 D M V K N Q A M A D A L E R F G E N R F 1780  
 5461 GTGTCACCAATTATCTGGACTTGTGGACAAACCTCTGCCATGGCCGTGGAAAGGG 5520  
 1781 V S P L S W D L V G R N L F A M A V E G 1800  
 5521 GTGGTGTCTCCTCATTACTGTCATGCCAGTACAGATTCTCATCAGGCCAGACCT 5580  
 1801 V V F F L I T V L I Q Y R F F I R P R P 1820  
 5581 GTAAATGCAAAGCTATCTCTCTGAATGATGAAGATGAAGATGTGAGGCGGAAAGACAG 5640  
 1821 V N A K L S P L N D E D E D V R R E R Q 1840  
 5641 AGAATTCTGTGGTGGAGGCCAGATGACATCTAGAAATCAAGGAGTTGACGGAAAGATA 5700  
 1841 R I L D G G G Q N D I L E I K E L T K I 1860  
 5701 TATAGAAGGAAGCGGAAGCCTGCTGTTGACAGGATTGCGCTGGGCATTCCCTGGTGAG 5760

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1861 Y R R K R K P A V D R I C V G I P P G E 1880  
 5761 TGCTTGGGCTCCGGAGTTAATGGGCTGAAAATCATCAACTTCAGATGTTAAC 5820  
 1881 C F G L L G V N G A G K S S T F K M L T 1900  
 5821 GGAGATAACACTGTACCGAGGGATGCCTTCCTAACAGAAAATAGTATCTTATCAAAC 5880  
 1901 G D T T V T R G D A F L N R N S I L S N 1920  
 5881 ATCCATGAAGTACATCAGAACATGGCTACTGCCCTCAGTTGATGCCATCACAGAGCTG 5940  
 1921 I H E V H Q N M G Y C P Q F D A I T E L 1940  
 5941 TTGACTGGGAGAGAACACGTGGAGTTGGCTTGAGAGGAGTCCCAGAGAAAGAA 6000  
 1941 L T G R E H V E F F A L L R G V P E K E 1960  
 6001 GTTGGCAAGGTTGGTAGTGGGCACTGGAAACTGGGCTCGTGAAGTATGGAGAAAAA 6060  
 1961 V G K V G E W A I R K L G L V K Y G E K 1980  
 6061 TATGCTGGTAACCTATAGTGAGGGACACAAACGGCAAGCTCTACAGCCATGSCTTTGATC 6120  
 1981 Y A G N Y S G G N K R K L S T A M A L I 2000  
 6121 CGGGGCTCCCTGGTGTCTGGATGAAACCCACAGGGCATGGATCCCAAAGCCCG 6180  
 2001 G G P P V V F L D E P T T G M D P K A R 2020  
 6181 CGGTTCTGTGGATTGTGCCCTAACGTCTGTCAAGGAGGGAGATCACTAGTGCTTACA 6240  
 2021 R F L W N C A L S V V K E G R S V V L T 2040  
 6241 TCTCATAGTATGGAGAATGTGAAGCTTTGCACTAGGATGGCAATCATGGCAATGG 6300  
 2041 S H S M E E C E A L C T R M A I M V N G 2060  
 6301 AGGTTCAAGGTGCTTGGCAGTGTCCAGCATCTAAAAAATAGGTTGGAGATGGTTATACA 6360  
 2061 R F R C L G S V Q H L K N R F G D G Y T 2080  
 6361 ATAGTTGACGAATAGCAGGGTCAACCCGGACCTGAAGCCCTGTCAGGATTCTTGG 6420  
 2081 I V V R I A G S N P D L K P V Q D F F G 2100  
 6421 CTTGCATTTCTGGAGTGTCCAAGAGAACACCGGAACATGCTACAATACCGCTT 6480  
 2101 L A F P G S V P K E K H R N M L Q Y Q L 2120  
 6481 CCATCTTCATTATCTCTGGCAGGATATTCAAGCATCTCTCCAGAGCAAAAGCGA 6540  
 2121 P S S L S S L A R I F S I L S Q S K K R 2140  
 6541 CTCCACATAGAAGACTACTCTGTCTCAGACAAACATTGACCAAGTATTGTGAAC 6600  
 2141 L H I E D Y S V S Q T T L D Q V F V N F 2160  
 6601 GCGAAGGACCAAAACTGTATGACCACTTAAAGACCTCTCATACACAAAAACAGACA 6660  
 2161 A K D Q S D D D H L K D L S L H K N Q T 2180  
 6661 GTAGTGGACGTTGCAGTCTCACATCTTCTACAGGATGAGAAAGTGAAGAAAGCT 6720  
 2181 V V D V A V L T S F L Q D E K V K E S Y 2200  
 6721 GTATGAAGAATCTGTTACAGGGGGCTGAAAGTAAAGAGGGACTAGACTTCTT 6780  
 2201 V \*  
 6781 GCACCATGTGAAGTGTGTGGAGAAAAGAGCCAGAACAGTTGATGTGGAGAAAGTAAACTG 6840  
 6841 GATACTGTACTGATACTATTCAATGCAATGCAATTCAATG 6880

Figure 3

5' 1 GTACCCCCCT TGCCTGGTTG ATCCTCAGGG TTCTACTTAG AATGCCTCGA

51 AAAGTCTTGG CTGGACACCC ATGCCAGTC TTTCTGCAGG GTCCCATATTG  
101 GGTAAACCTT CTCATTCTAT CCCATGTGAA CCAGGCCAGG CCCATCAGGG  
151 TTGGCAACC CCCTGTGCA GTGGTTGCTG CCAGGTGACA GGAGCAAGCC  
201 TGCACTGCT GGGGGCCAT CGAGAGACAG CCTGCCAGAG GGGAGAACAC  
251 CTGGGAGGC CAGAGCGTG GAGACAGCAA GAGACCAGGG GCTGAGGACA  
301 GAGTAGTACA GGTCTTGGT CCCAGTAGTC CTGAAACACAC TGCACCTCCGA  
351 ACCTTTCTGT ACTTAGCTTA AGCCAGTTGG AGTTTCTGTC CTTTACAACC  
401 AAGAGCTTG ATAGGAATGG GGTCTCTGTC TAGCTGACTG TTGGCTCTT  
451 TCCCGATCGG CGCTGGAGG GGAACACAGC AGTGACTACA GTGGGTAGCT  
501 TACTCGGTGCG TGGCGATGCT AGAAAGTGTG TGCCATGCTT TATTTCCCAC  
551 GTGGTGGGGAA TTTTGACCCCC ACCTGTACAG ACAGATAAAGT GAGGACCCCTT  
601 TTCACCTTAT CCTGCAACAG AAAATCCAGC AGCCAAAGCC AACAAAGGGCC  
651 CAGCATAGCA TCTCCCTCT CTGACTTCAT CCTCACGCTC CACACACCAT  
701 CCCCCCTGGCC ATTCCCAGCA GCCCAGTAAG CACTGCCTCA CACTTCCAGT  
751 TCCGGACCCAG CCAGGATGGC CAGGCTGTGAT GGGGGCCATC CACCGGCTGA  
801 AGCCAATTCG CTATTCCTGA GCTGAAGGTG AATCAATCCC GCATAAAATCT  
851 TCAGGGACAGAG AACTNGGGTG GGGGGTAGAA GAGGGGGAT GTCTAGAAGG  
901 AAATTCTGGG GCACATTCCCT GGAAAGTGGAGG AGGATGGATA TTGGACAGAAA  
951 ATTATGTCAT TGCAAGGCACCT CTCACTTGGCC CTGGCCACAT GGCAAGTTCC  
1001 TCCCCGGCTG TGTTCCGNNC CTCTCTGCTG GCTCCAGGGC CTGTCCTGTC  
1051 CTGGAGCGAG ATGGGTCCCA GGGCTGGCA CCAGTCCCCA TCTCCAGCCA  
1101 TCAGGCACCTT TCCTCTGCTG GTTTGGGT AAACACNTCC CTAGGTTGG  
1151 GGATCTGAAT CCTCTCTCCA ACACACTCAA GCCTTGCTGG GCCTCCCTGC  
1201 AGTGTATGTT TAAGGCACCA CACAGCCTCC AAGGCCTGGC ACCCGGGCAG  
1251 TGCCCACCTG GTAAACACAG CAGTCAGATT TCCTCATTTC AGCCAAGTGT  
1301 AAAATCAAGG TAATGGATAC ACNTTTTTT TTTTNTNTT TTTCCAGGGG  
1351 GNTNNNTTTT TTTGAGAGC GAGTCCTACT CTGTCANCCC CGGCTCTGGAG  
1401 TGCACTGGCT CAATCTGGC TCANCTGGCA AGTCCCGCT CCCAGGTTCA  
1451 TGCCATTCTC CTGCTCTGAGC CTACATAGTA GCTGGGACTA CAGGTGCCG  
1501 CCACCACACCC TAGCTAATTT TTTGTATTT TAGTAGAGAC GGGGGTTCTA  
1551 CATGTTAGCC AGGATGGCT CGATCTCTG ACCTCCAAA GTGGTGGGAG  
1601 TTACAGGTTG GAGCCACTGC GCNCCGGCTG GATGACTCTT GAGACAACAC  
1651 CATTCAAGACA AAGGCAGGC CTCCCACCTTA AACTCATAAC CGTGTCTCCT  
1701 TTCTCTCTT CGATTTGAGC GGCTGAATTG GTTACAGTC ATCTGACCTG  
1751 TGGGTGTGAA NGTCCACCTG CCTGGCATAA AAAGCTGTG CTCCTTCTA  
1801 GGTGAGGAGA AAGAGAGAGA CCTGGCTCAT CTGAGGTGTG GTGGGAGGG  
1851 GGGACCCAGG TGTGCTGGAA ATGAAAAGAA ATGCATTCCT GTTTTCTCGT  
1901 CCCAACATGC AAACAACCTGA ACAAAAGCAT TAGGGCTGA GACTGGGAGT  
1951 AAAGAATTCC TTGTACCCAT GGATACCCAGG AAATGCCCACTTATATAT  
2001 ATAAGGGCT TTAGAGATGC TGACCATCT GATATTCCAG CCTGGGGCCA  
2051 CATGGGAGTG TGCCCTGGT TTATTCCTTA TACAGTTCCA TGAACATGGC  
2101 TCTGGAAACA CCTCTGTCTG CAGAAATGA GGCTTTCTT TTTTGTCTG

2151 GGGGTGAACA GAGGGCAGAG GCCTGGGCAT CTTCACTCAG CACCCCTTG  
 2201 TAACCCAGCA CTTAGCACCA TGGCTGGCC ACAGCAATGT CACATGTG  
 2251 AGTGCACACG ATGCCCTACT GCCAGGGTC ACCCCACACC GGTGCTGTTG  
 2301 GGGCGTTGG AGTGGTATC TCTTCTTAG TCCTCAAGCT CCTACCTGGC  
 2351 AGAGAGCTGC CCAACACCGT CGGGGTGGGG TGGGCGGGAA GGGAAAGAC  
 2401 AGCAGCAAGA AAGAACCCCCC CTGGCCCTCA CTCTCCCTCC CTGGACGCC  
 2451 CCTCTTGAC CCCATCACAC AGCCGCTTGA GCCTGGAGN CAGTGGATT  
 2501 CCAGCGCTGG GAACCCCCCCC CGTCTGTCCC GTGTCCCCC GGAGCCTCAC  
 2551 CCNCGTGCTC CGGCAGCCCC CGCGAGTTCG GGACCCGGGG TTTCGGGGT  
 2601 GGCAGGGGGT TCCCATGCCG CCTGGCAGGC CTGGCTCGG GCCGCTCCC  
 2651 GAACCTGAC TTCAGGGGTC CTGGCTGCCG GCCCCAGCA GGAGCAAAC  
 2701 AAGAGCACCG GCACCTGCCG GCCCGCCCG CCCCTTGGT CCGGCCAATC  
 2751 GCGCGCTGG GGCGGGGTCG GGCGCGCTGG AACAGAGCC GGAGCCGGAT  
 2801 CCCAGCCGGA GCCCAAGCGC AGCCCGCACC CGCGCAGCG GCTGAGCCGG  
 2851 GAGCCAGGGC AGCCCTGCCG CCCAGCTCA AGCCTGCTCC CGCCCGCCNG  
 2901 CCGCCGACG CGCCGCCCG CGCCCCCGGG GCATGGCTGT CTGATGCCG

## EXON1/INTRON 1

2951 CTTCTCGGT CGGCACCGGG ATGGTGAAGTG AGCGCATCCT TCGTCCGCCG  
 3001 GGAACGTTT TATTTCAAG GAGAGCAGGA AACACACAAA GACTCGCAAG  
 3051 CTCGACCTGA CACCCCTCCC AGGAGCGCGT CCTCTGGGGC GCTGACCCAG  
 3101 GGGCACCTTA GAGTGGCGCC CGGCTCCGAT CGCTGCCCT NNCCCTCCG  
 3151 CCAGGCCAC CTGGGAGCCT CGGGGATGCC CCTTGACCCG GCAGAGNGCA  
 3201 CGGACTAGGT GGAGGGNNCC GGGATTGGGG CGGGGGGCAG NCAGTTGCC  
 3251 TACAAGTGG ACCGATGGCC TTGACCTGAT GGCTTCTGGG CGGGGGGGT  
 3301 GGGGAGCTGG GGACCCGGG CGCACCTGGG ACTGGGGAGG GGCGCCAGCT  
 3351 TGGGCCGAG GGAAGAGGGG ACTTGAGAA GGGGAGCCCC GCGCGCGGG  
 3401 CTGTGGGCTT GGGGACCCGG GACTTCGCG GCCATCCCCA GGAAACGCCAG  
 3451 GCAAGGTCTG GGGAACAAAA GAGGAAGCTG CCCCCAGAGA GCCGGAGCTC  
 3501 GACTGNACTC CC 3'

Figure 4

5'

1 CTTGGTGCCG CATGCATCGT GTTGCTCATC TTTCTGGCCT TCCAGCAGAG  
 51 GGCATATGTC GCCCCTGCCA ACCTGCCTGC TCTCTGCTG TTGCTACTAC  
 101 TGATGGCTG GTGATCACA CCGCTCATGT ACCCAGCCTC CTTCTCTTC  
 151 TCCGTGCCCA GCACACCTTA TGTGGTGCCTC ACCTGCATAA ACCTCTTTAT  
 201 TGGCATCAAT GGAAGCATGG CCACCTTGTG GCTTGAGCTC TTCTCTGATC  
 251 AGAAGCTGCA GGAGGTGAGC CGGATCTGA AACAGGTCTT CTTATCTTC  
 301 CCCACTCTG CTTGGCCGG GGGCTTATTG ACATGGTGC GNAACCAGGC  
 351 CATGGCTGAT GCCTTGTGANC CTTGGAAAG AAGGCAGTTC AAGTACCTG

401 NCTTGGAAAGG TGGCGGAAGA ACCTTTGGC ATGGGAACAG GCCCCCTTT  
451 CCTTCTCTTC ACACTANTGT TCAAGCACCG AAGCCAACTC NTGCCACAAG  
501 CCCAGGTAAG GTCTCTGCCA CTCCGGAGA GAGACGAGGA TGTAGCCCGT  
551 GAACGGGAGC GGGTGGTCCA AGGAGGCCACC CAGGGGGATG TGTGGTGCT  
601 GAGGAACATTG ACCAAGGTAT ACCGTGGGCA GAGGATGCCA GCTGTTGACC  
651 GCTTGTGCCT GGGGATTCCC CCTGGTGAAGT GTTTGGGCT GTGGGTGTG  
701 AACGGAGGAG GGAAGACCTC CACGTTTCGC ATGGTACGG GGGACACATT  
751 GCCCAGCAGG GGGCAGGGCTG TGCTGGCAGG CCACACGGG CCGGGAAACC  
801 CAGTGTGCGC ACCTCNAGGG CAGGCNCAGC GTGGCCCGGG AACCCAGTGC  
851 TGCGCACCTA AGCATGGGAT ACTGCCCTNA ATCCGATGCC ATCTTGAGC  
901 TGCTGACGGG CGGGAGCAC CTGGAGCTGC TTGGGGCCCT GCGCGGTGTC  
951 CGGGAGGCC AGGTTGCCA NACCGNTGGC TCGGGCCTGG CGCGTCTGGG  
1001 ACTCTCATGG TACGCAGACC GGCTGCAGG CACCTACAGG AACCTGCCCG  
1051 GGCGGCCGCT CGAGCCNTA NNTGAAGTA 3'

Figure 4b

...CTCCTGCCAC AGTTAGTGAG GTCTATGGAG AGGGTGGCAG GGGCCAAGGA  
CCTACTTTAA GCCCACAGAT ATTCTGTCCC CAGGCCAGG GTGAGGTCTC...

Figure 5

*CDNA-sequences of lipid sensitive Genes:*

*ABCB9, ABCA6, ABCC4, ABCA1, ABCD2, ABCB1, ABCB4, ABCC2, ABCD1, ABCC1, ABCB6, ABCB11, ABCG2, ABCC5, ABCA5, ABCG1, ABCA3*

*ABCB9 GENBANK:U66676*

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*ABCA6 GENBANK:U66680*

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ABCA5 Acc.Nr.: AF000148 GENBANK:HSAF000148

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ABCG1 Acc.Nr.: U34919 GENBANK: HSU34919

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## Fragment 640918

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## Huwhite2

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**COMBINED DECLARATION AND POWER OF ATTORNEY**

ATTORNEY DOCKET NO

a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**ATP BINDING CASSETTE GENES AND PROTEINS FOR DIAGNOSIS AND TREATMENT OF LIPID DISORDERS AND INFLAMMATORY DISEASES**

the specification of which is attached hereto,

or was filed on **March 25, 2001**

as a PCT Application Serial No. PCT/EP99/06991 *U.S. SERIAL NO. 09/786,635  
FILED MARCH 7, 2001*

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s), the priority(ies) of which is/are to be claimed:

**60/101,706**                   **USA**                   **September 25, 1998**  
(Number)                   (Country)                   (Month/Day/Year Filed)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose the material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)

(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**POWER OF ATTORNEY:** As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Kurt G. Briscoe, Reg. No. 33,141; William C. Gerstenzang, Reg. No. 27,552 and Stephen G. Ryan, Reg. No. 39,015, all of 220 East 42nd Street, 30th Floor, New York, New York 10017, and William R. Robinson, Reg. No. 27,224.

Davy E. Zoneraich, Reg. No. 37,267 and Mark A. Montana, Reg. No. 44,948, all of 721 Route 202-206, Bridgewater, New Jersey 08807, my attorneys with full power of substitution and revocation

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--	--

FULL NAME OF SOLE OR FIRST INVENTOR <b>Gerd Schmitz</b>	INVENTOR'S SIGNATURE <i>Gerd Schmitz</i>	DATE <b>10.3.2001</b>
RESIDENCE <b>D 93161 Sinzing, Germany</b>	CITIZENSHIP <b>German</b>	
POST OFFICE ADDRESS <b>Turmstr. 15a, D 93161 Sinzing, Germany</b>		
FULL NAME OF SECOND INVENTOR <b>Jochen Klucken</b>	INVENTOR'S SIGNATURE <i>Jochen Klucken</i>	DATE <b>10.3.2001</b>
RESIDENCE <b>D 93047 Regensburg, Germany</b>	CITIZENSHIP <b>German</b>	
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FULL NAME OF THIRD INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FOURTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
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## SEQUENCE LISTING

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gcaccatgtt gaaatgttgc gaaaaatggggcc gtcacccgtt gatgtggaaag aagtaacttgc 6840  
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&lt;211&gt; 2201

<212> PRT

<213> Human

<220>

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15

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20

25

30

Val Val Gly Asn Phe Asn Lys Ser Ile Val Ala Arg Leu Phe Ser Asp

35

40

45

Ala Arg Arg Leu Leu Leu Tyr Ser Gln Lys Asp Thr Ser Met Lys Asp

50

55

60

Met Arg Lys Val Leu Arg Thr Leu Gln Gln Ile Lys Lys Ser Ser Ser

65

70

75

80

Asn Leu Lys Leu Gln Asp Phe Leu Val Asp Asn Glu Thr Phe Ser Gly

85

90

95

Phe Leu Tyr His Asn Leu Ser Leu Pro Lys Ser Thr Val Asp Lys Met

100

105

110

Leu Arg Ala Asp Val Ile Leu His Lys Val Phe Leu Gln Gly Tyr Gln

115

120

125

Leu His Leu Thr Ser Leu Cys Asn Gly Ser Lys Ser Glu Glu Met Ile

130

135

140

Gln Leu Gly Asp Gln Glu Val Ser Glu Leu Cys Gly Leu Pro Arg Glu

145

150

155

160

T03250-5E998760

Lys Leu Ala Ala Ala Glu Arg Val Leu Arg Ser Asn Met Asp Ile Leu  
165 170 175

Lys Pro Ile Leu Arg Thr Leu Asn Ser Thr Ser Pro Phe Pro Ser Lys  
180 185 190

Glu Leu Ala Glu Ala Thr Lys Thr Leu Leu His Ser Leu Gly Thr Leu  
195 200 205

Ala Gln Glu Leu Phe Ser Met Arg Ser Trp Ser Asp Met Arg Gln Glu  
210 215 220

Val Met Phe Leu Thr Asn Val Asn Ser Ser Ser Ser Thr Gln Ile  
225 230 235 240

Tyr Gln Ala Val Ser Arg Ile Val Cys Gly His Pro Glu Gly Gly Gly  
245 250 255

Leu Lys Ile Lys Ser Leu Asn Trp Tyr Glu Asp Asn Asn Tyr Lys Ala  
260 265 270

Leu Phe Gly Gly Asn Gly Thr Glu Glu Asp Ala Glu Thr Phe Tyr Asp  
275 280 285

Asn Ser Thr Thr Pro Tyr Cys Asn Asp Leu Met Lys Asn Leu Glu Ser  
290 295 300

Ser Pro Leu Ser Arg Ile Ile Trp Lys Ala Leu Lys Pro Leu Leu Val  
305 310 315 320

Gly Lys Ile Leu Tyr Thr Pro Asp Thr Pro Ala Thr Arg Gln Val Met  
325 330 335

Ala Glu Val Asn Lys Thr Phe Gln Glu Leu Ala Val Phe His Asp Leu  
340 345 350

Glu Gly Met Trp Glu Glu Leu Ser Pro Lys Ile Trp Thr Phe Met Glu

002250-000998769

355                    360                    365

Asn Ser Gln Glu Met Asp Leu Val Arg Met Leu Leu Asp Ser Arg Asp  
370                    375                    380

Asn Asp His Phe Trp Glu Gln Gln Leu Asp Gly Leu Asp Trp Thr Ala  
385                    390                    395                    400

Gln Asp Ile Val Ala Phe Leu Ala Lys His Pro Glu Asp Val Gln Ser  
405                    410                    415

Ser Asn Gly Ser Val Tyr Thr Trp Arg Glu Ala Phe Asn Glu Thr Asn  
420                    425                    430

Gln Ala Ile Arg Thr Ile Ser Arg Phe Met Glu Cys Val Asn Leu Asn  
435                    440                    445

Lys Leu Glu Pro Ile Ala Thr Glu Val Trp Leu Ile Asn Lys Ser Met  
450                    455                    460

Glu Leu Leu Asp Glu Arg Lys Phe Trp Ala Gly Ile Val Phe Thr Gly  
465                    470                    475                    480

Ile Thr Pro Gly Ser Ile Glu Leu Pro His His Val Lys Tyr Lys Ile  
485                    490                    495

Arg Met Asp Ile Asp Asn Val Glu Arg Thr Asn Lys Ile Lys Asp Gly  
500                    505                    510

Tyr Trp Asp Pro Gly Pro Arg Ala Asp Pro Phe Glu Asp Met Arg Tyr  
515                    520                    525

Val Trp Gly Gly Phe Ala Tyr Leu Gln Asp Val Val Glu Gln Ala Ile  
530                    535                    540

Ile Arg Val Leu Thr Gly Thr Glu Lys Lys Thr Gly Val Tyr Met Gln  
545                    550                    555                    560

002250-55998760

Gln Met Pro Tyr Pro Cys Tyr Val Asp Asp Ile Phe Leu Arg Val Met  
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Ser Arg Ser Met Pro Leu Phe Met Thr Leu Ala Trp Ile Tyr Ser Val  
580 585 590

Ala Val Ile Ile Lys Gly Ile Val Tyr Glu Lys Glu Ala Arg Leu Lys  
595 600 605

Glu Thr Met Arg Ile Met Gly Leu Asp Asn Ser Ile Leu Trp Phe Ser  
610 615 620

Trp Phe Ile Ser Ser Leu Ile Pro Leu Leu Val Ser Ala Gly Leu Leu  
625 630 635 640

Val Val Ile Leu Lys Leu Gly Asn Leu Leu Pro Tyr Ser Asp Pro Ser  
645 650 655

Val Val Phe Val Phe Leu Ser Val Phe Ala Val Val Thr Ile Leu Gln  
660 665 670

Cys Phe Leu Ile Ser Thr Leu Phe Ser Arg Ala Asn Leu Ala Ala Ala  
675 680 685

Cys Gly Gly Ile Ile Tyr Phe Thr Leu Tyr Leu Pro Tyr Val Leu Cys  
690 695 700

Val Ala Trp Gln Asp Tyr Val Gly Phe Thr Leu Lys Ile Phe Ala Ser  
705 710 715 720

Leu Leu Ser Pro Val Ala Phe Gly Phe Gly Cys Glu Tyr Phe Ala Leu  
725 730 735

Phe Glu Glu Gln Gly Ile Gly Val Gln Trp Asp Asn Leu Phe Glu Ser  
740 745 750

Y02250-52998760

Pro Val Glu Glu Asp Gly Phe Asn Leu Thr Thr Ser Val Ser Met Met  
755 760 765

Leu Phe Asp Thr Phe Leu Tyr Gly Val Met Thr Trp Tyr Ile Glu Ala  
770 775 780

Val Phe Pro Gly Gln Tyr Gly Ile Pro Arg Pro Trp Tyr Phe Pro Cys  
785 790 795 800

Thr Lys Ser Tyr Trp Phe Gly Glu Glu Ser Asp Glu Lys Ser His Pro  
805 810 815

Gly Ser Asn Gln Lys Arg Ile Ser Glu Ile Cys Met Glu Glu Glu Pro  
820 825 830

Thr His Leu Lys Leu Gly Val Ser Ile Gln Asn Leu Val Lys Val Tyr  
835 840 845

Arg Asp Gly Met Lys Val Ala Val Asp Gly Leu Ala Leu Asn Phe Tyr  
850 855 860

Glu Gly Gln Ile Thr Ser Phe Leu Gly His Asn Gly Ala Gly Lys Thr  
865 870 875 880

Thr Thr Met Ser Ile Leu Thr Gly Leu Phe Pro Pro Thr Ser Gly Thr  
885 890 895

Ala Tyr Ile Leu Gly Lys Asp Ile Arg Ser Glu Met Ser Thr Ile Arg  
900 905 910

Gln Asn Leu Gly Val Cys Pro Gln His Asn Val Leu Phe Asp Met Leu  
915 920 925

Thr Val Glu Glu His Ile Trp Phe Tyr Ala Arg Leu Lys Gly Leu Ser  
930 935 940

Glu Lys His Val Lys Ala Glu Met Glu Gln Met Ala Leu Asp Val Gly

T0225D-SEC98/60

945	950	955	960
Leu Pro Ser Ser Lys Leu Lys Ser Lys Thr Ser Gln Leu Ser Gly Gly			
965	970	975	
Met Gln Arg Lys Leu Ser Val Ala Leu Ala Phe Val Gly Gly Ser Lys			
980	985	990	
Val Val Ile Leu Asp Glu Pro Thr Ala Gly Val Asp Pro Tyr Ser Arg			
995	1000	1005	
Arg Gly Ile Trp Glu Leu Leu Leu Lys Tyr Arg Gln Gly Arg Thr Ile			
1010	1015	1020	
Ile Leu Ser Thr His His Met Asp Glu Ala Asp Val Leu Gly Asp Arg			
1025	1030	1035	1040
Ile Ala Ile Ile Ser His Gly Lys Leu Cys Cys Val Gly Ser Ser Leu			
1045	1050	1055	
Phe Leu Lys Asn Gln Leu Gly Thr Gly Tyr Tyr Leu Thr Leu Val Lys			
1060	1065	1070	
Lys Asp Val Glu Ser Ser Leu Ser Ser Cys Arg Asn Ser Ser Ser Thr			
1075	1080	1085	
Val Ser Tyr Leu Lys Lys Glu Asp Ser Val Ser Gln Ser Ser Ser Asp			
1090	1095	1100	
Ala Gly Leu Gly Ser Asp His Glu Ser Asp Thr Leu Thr Ile Asp Val			
1105	1110	1115	1120
Ser Ala Ile Ser Asn Leu Ile Arg Lys His Val Ser Glu Ala Arg Leu			
1125	1130	1135	
Val Glu Asp Ile Gly His Glu Leu Thr Tyr Val Leu Pro Tyr Gly Al			
1140	1145	1150	

Ala Lys Glu Gly Ala Phe Val Glu Leu Phe His Glu Ile Asp Asp Arg  
1155 1160 1165

Leu Ser Asp Leu Gly Ile Ser Ser Tyr Gly Ile Ser Glu Thr Thr Leu  
1170 1175 1180

Glu Glu Ile Phe Leu Lys Val Ala Glu Glu Ser Gly Val Asp Ala Glu  
1185 1190 1195 1200

Thr Ser Asp Gly Thr Leu Pro Ala Arg Arg Asn Arg Arg Ala Phe Gly  
1205 1210 1215

Asp Lys Gln Ser Cys Leu Arg Pro Phe Thr Glu Asp Asp Ala Ala Asp  
1220 1225 1230

Pro Asn Asp Ser Asp Ile Asp Pro Glu Ser Arg Glu Thr Asp Leu Leu  
1235 1240 1245

Ser Gly Met Asp Gly Lys Gly Ser Tyr Gln Val Lys Gly Trp Lys Leu  
1250 1255 1260

Thr Gln Gln Gln Phe Val Ala Leu Leu Trp Lys Arg Leu Leu Ile Ala  
1265 1270 1275 1280

Arg Arg Ser Arg Lys Gly Phe Phe Ala Gln Ile Val Leu Pro Ala Val  
1285 1290 1295

Phe Val Cys Ile Ala Leu Val Phe Ser Leu Ile Val Pro Pro Phe Gly  
1300 1305 1310

Lys Tyr Pro Ser Leu Glu Leu Gln Pro Trp Met Tyr Asn Glu Gln Tyr  
1315 1320 1325

Thr Phe Val Ser Asn Asp Ala Pro Glu Asp Thr Gly Thr Leu Glu Leu  
1330 1335 1340

Leu Asn Ala Leu Thr Lys Asp Pro Gly Phe Gly Thr Arg Cys Met Glu  
1345                    1350                    1355                    1360

Gly Asn Pro Ile Pro Asp Thr Pro Cys Gln Ala Gly Glu Glu Trp  
1365                    1370                    1375

Thr Thr Ala Pro Val Pro Gln Thr Ile Met Asp Leu Phe Gln Asn Gly  
1380                    1385                    1390

Asn Trp Thr Met Gln Asn Pro Ser Pro Ala Cys Gln Cys Ser Ser Asp  
1395                    1400                    1405

Lys Ile Lys Lys Met Leu Pro Val Cys Pro Pro Gly Ala Gly Gly Leu  
1410                    1415                    1420

Pro Pro Pro Gln Arg Lys Gln Asn Thr Ala Asp Ile Leu Gln Asp Leu  
1425                    1430                    1435                    1440

Thr Gly Arg Asn Ile Ser Asp Tyr Leu Val Lys Thr Tyr Val Gln Ile  
1445                    1450                    1455

Ile Ala Lys Ser Leu Lys Asn Lys Ile Trp Val Asn Glu Phe Arg Tyr  
1460                    1465                    1470

Gly Gly Phe Ser Leu Gly Val Ser Asn Thr Gln Ala Leu Pro Pro Ser  
1475                    1480                    1485

Gln Glu Val Asn Asp Ala Thr Lys Gln Met Lys Lys His Leu Lys Leu  
1490                    1495                    1500

Ala Lys Asp Ser Ser Ala Asp Arg Phe Leu Asn Ser Leu Gly Arg Phe  
1505                    1510                    1515                    1520

Met Thr Gly Leu Asp Thr Arg Asn Asn Val Lys Val Trp Phe Asn Asn  
1525                    1530                    1535

Lys Gly Trp His Ala Ile Ser Ser Phe Leu Asn Val Ile Asn Asn Ala

T0225D-SEG9B/260

102250-5C998Z60

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Ile Leu Arg Ala Asn Leu Gln Lys Gly Glu Asn Pro Ser His Tyr Gly		
1555	1560	1565
Ile Thr Ala Phe Asn His Pro Leu Asn Leu Thr Lys Gln Gln Leu Ser		
1570	1575	1580
Glu Val Ala Pro Met Thr Thr Ser Val Asp Val Leu Val Ser Ile Cys		
1585	1590	1595
1600		
Val Ile Phe Ala Met Ser Phe Val Pro Ala Ser Phe Val Val Phe Leu		
1605	1610	1615
Ile Gln Glu Arg Val Ser Lys Ala Lys His Leu Gln Phe Ile Ser Gly		
1620	1625	1630
Val Lys Pro Val Ile Tyr Trp Leu Ser Asn Phe Val Trp Asp Met Cys		
1635	1640	1645
Asn Tyr Val Val Pro Ala Thr Leu Val Ile Ile Ile Phe Ile Cys Phe		
1650	1655	1660
Gln Gln Lys Ser Tyr Val Ser Ser Thr Asn Leu Pro Val Leu Ala Leu		
1665	1670	1675
1680		
Leu Leu Leu Tyr Gly Trp Ser Ser Ile Thr Pro Leu Met Tyr Pro Ala		
1685	1690	1695
Ser Phe Val Phe Lys Ile Pro Ser Thr Ala Tyr Val Val Leu Thr Ser		
1700	1705	1710
Val Asn Leu Phe Ile Gly Ile Asn Gly Ser Val Ala Thr Phe Val Leu		
1715	1720	1725
Glu Leu Phe Thr Asp Asn Lys Leu Asn Asn Ile Asn Asp Ile Leu Lys		
1730	1735	1740

Ser Val Phe Leu Ile Phe Pro His Phe Cys Leu Gly Arg Gly Leu Ile  
1745 1750 1755 1760

Asp Met Val Lys Asn Gln Ala Met Ala Asp Ala Leu Glu Arg Phe Gly  
1765 1770 1775

Glu Asn Arg Phe Val Ser Pro Leu Ser Trp Asp Leu Val Gly Arg Asn  
1780 1785 1790

Leu Phe Ala Met Ala Val Glu Gly Val Val Phe Phe Leu Ile Thr Val  
1795 1800 1805

Leu Ile Gln Tyr Arg Phe Phe Ile Arg Pro Arg Pro Val Asn Ala Lys  
1810 1815 1820

Leu Ser Pro Leu Asn Asp Glu Asp Glu Asp Val Arg Arg Glu Arg Gln  
1825 1830 1835 1840

Arg Ile Leu Asp Gly Gly Gln Asn Asp Ile Leu Glu Ile Lys Glu  
1845 1850 1855

Leu Thr Lys Ile Tyr Arg Arg Lys Arg Lys Pro Ala Val Asp Arg Ile  
1860 1865 1870

Cys Val Gly Ile Pro Pro Gly Glu Cys Phe Gly Leu Leu Gly Val Asn  
1875 1880 1885

Gly Ala Gly Lys Ser Ser Thr Phe Lys Met Leu Thr Gly Asp Thr Thr  
1890 1895 1900

Val Thr Arg Gly Asp Ala Phe Leu Asn Arg Asn Ser Ile Leu Ser Asn  
1905 1910 1915 1920

Ile His Glu Val His Gln Asn Met Gly Tyr Cys Pro Gln Phe Asp Ala  
1925 1930 1935

T02250 \* S2998760

Ile Thr Glu Leu Leu Thr Gly Arg Glu His Val Glu Phe Phe Ala Leu  
1940 1945 1950

Leu Arg Gly Val Pro Glu Lys Glu Val Gly Lys Val Gly Glu Trp Ala  
 1955 1960 1965

Ile Arg Lys Leu Gly Leu Val Lys Tyr Gly Glu Lys Tyr Ala Gly Asn  
 1970 1975 1980

Tyr Ser Gly Gly Asn Lys Arg Lys Leu Ser Thr Ala Met Ala Leu Ile  
 1985                  1990                  1995                  2000

Gly Gly Pro Pro Val Val Phe Leu Asp Glu Pro Thr Thr Gly Met Asp  
2005 2010 2015

Pro Lys Ala Arg Arg Phe Leu Trp Asn Cys Ala Leu Ser Val Val Lys  
2020 2025 2030

Glu Gly Arg Ser Val Val Leu Thr Ser His Ser Met Glu Glu Cys Glu  
2035 2040 2045

Ala Leu Cys Thr Arg Met Ala Ile Met Val Asn Gly Arg Phe Arg Cys  
2050 2055 2060

Leu Gly Ser Val Gln His Leu Lys Asn Arg Phe Gly Asp Gly Tyr Thr  
 2065 2070 2075 2080

Ile Val Val Arg Ile Ala Gly Ser Asn Pro Asp Leu Lys Pro Val Gln  
2085 2090 2095

Asp Phe Phe Gly Leu Ala Phe Pro Gly Ser Val Pro Lys Glu Lys His  
2100 2105 2110

Arg Asn Met Leu Gln Tyr Gln Leu Pro Ser Ser Ser Leu Ser Ser Leu Ala  
2115 2120 2125

Arg Ile Phe Ser Ile Leu Ser Gln Ser Lys Lys Arg Leu His Ile Glu

2130

2135

2140

Asp Tyr Ser Val Ser Gln Thr Thr Leu Asp Gln Val Phe Val Asn Phe  
2145 2150 2155 2160

Ala Lys Asp Gln Ser Asp Asp Asp His Leu Lys Asp Leu Ser Leu His  
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Lys Asn Gln Thr Val Val Asp Val Ala Val Leu Thr Ser Phe Leu Gln  
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<212> PRT

<213> Human

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<223> Partial peptide sequence of ABCG1 (ABC8)

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Ser Tyr Val Arg Tyr Gly Phe Glu Gly Val Ile Leu Ser Ile Tyr Gly  
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Leu Asp Arg Glu Asp Leu His Cys Asp Ile Asp Glu Thr Cys His Phe  
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Gln Lys Ser Glu Ala Ile Leu Arg Glu Leu Asp Val Glu Asn Ala Lys  
50 55 60

Leu

65

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<220>

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<213> Human

<220>

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<213> Human

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<211> 2911

<212> DNA

<213> Human

<220>

<223> human cDNA of ABCA8 (ABC-new)

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<213> Human

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2258

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<213> Human

53307

22202

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&lt;400&gt; 53

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&lt;210&gt; 54

&lt;211&gt; 2923

&lt;212&gt; DNA

&lt;213&gt; Human

&lt;220&gt;

&lt;223&gt; human genomic DNA of 5'-UTR of ABCG1

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